CRANFIELD UNIVERSITY

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THE ANALYSIS OF LATENT FINGERMARK CHEMISTRY USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY

CRANFIELD DEFENCE AND SECURITY

PhD
Academic Year: 2017- 2018

Supervisor: Professor Keith Rogers
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ABSTRACT

Latent fingermarks are comprised of a complex mixture of organic and inorganic components that exhibit broad chemical variability. Fingermarks are dynamic compositions prone to degradation over time and in varying environmental conditions. The complexity of latent fingermark chemistry has led to an abundance of literature over a number of years utilising various analytical techniques, which have endeavoured to provide a greater understanding of these complex chemical systems. In particular, a key focus has been on fingermark decomposition, and with recent advances in analytical instrumentation a more in-depth understanding of the dynamics of fingermark chemistry has been achieved, yet despite this, there remain significant gaps in the literature.

The work presented within this thesis looks at various aspects of latent fingermark chemistry that aim to address these gaps. During this research, the capabilities and limitations of Fourier-Transform Infrared (FTIR) spectromicroscopy were compared to the more established analytical technique of gas chromatography-mass spectrometry for the analysis of latent fingermarks. A novel approach to analysing the changes in latent fingermark chemistry over time at various moderate temperatures was demonstrated. An investigation into the intermolecular interactions of lipid components within simplified analogue 'fingermark' solutions was conducted, and the implications of these interactions for natural fingermark chemistry considered. Finally, the temporal degradation of illicit substances in latent fingermarks using spectroscopic imaging was investigated.

The results of this study, structured in the form of four research papers, demonstrate the complexity of latent fingermark composition, variability, and analysis. The use of FTIR spectromicroscopy to study *in-situ*, real-time changes in fingermark chemistry subjected to varying temperatures showed that total composition is affected by temperatures above 55^{o}C, and oxidation mechanisms take place almost immediately after deposition, even at room temperature. The use of simplified analogue 'fingermark' solutions to study intermolecular

interactions within natural fingermarks identified two key components, squalene and cholesterol, that potentially affect downstream organic interactions post-deposition. Finally, spectroscopic imaging successfully identified and spatially mapped aged illicit substances present within latent fingermarks up to thirty days' post-deposition. It was also possible to quantify the degradation of those illicit compounds over time. Due to the different facets of this research, the results of this thesis are expected to have an impact on a broad range of disciplines both within academia and for more practical forensic applications.

Keywords

Latent fingermark, FTIR spectroscopy, degradation, oxidation, contaminants, decomposition, aging, imaging, detection

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LIST OF ABBREVIATIONS

AN	Ammonium Nitrate
ATR	Attenuated Total Reflectance
C	Cocaine
CHCA	α-cyano-4-hydroxycinnamic acid
CN	Chlorononane
CSI	Crime Scene Investigation
ESI-MS	Electronspray Ionisation-Mass spectrometry
FA	Fatty Acid
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography-Mass Spectrometry
IED	Improvised Explosive Device
IFRG	International Fingerprint Research Group
IMS	Ion Mobility Spectroscopy
MALDI	Matrix Assisted Laser Desorption/Ionization
MCT	Mercury Cadmium Telluride
MS	Mass Spectrometry
NIST	National Institute of Standards and Technology
PETN	Pentaerythritol Tetranitrate
R&D	Research & Development
RDX	Research Development Explosive/ Cyclotrimethylenetrinitramine
SALDI	Surface Assisted laser desorption/Ionisation
SC	Sodium Chlorate
SERS	Surface Enhanced Raman Spectroscopy
SOCO	Scenes of Crime Officer
SOP	Standard Operating Procedure
SQ	Squalene
TNB	Trinitrobenzene
TNT	Trinitrotoluene
TOF	Time of Flight
TRL	Technology Readiness Level

CHAPTER 1. THESIS STRUCTURE

The thesis is organised in the research paper format. Each core chapter is arranged in the form of a journal article, two of which have been published in peer reviewed journals, and two are currently under review. Table 1 demonstrates the structure of the thesis in detail. The reason for adopting this approach rather than a more traditional, monographic PhD thesis is that the author deemed this structure more suitable given the independent nature of each objective. Each objective demonstrated original research the author felt worthy of publication, but when combined these papers provide a broad and comprehensive overview of the capabilities of FTIR spectroscopy as an analytical tool for the analysis of latent fingermarks. The author also felt that, with the increased pressure on all academics to publish, the value of this style of thesis as a learning tool for the preparation and submission of peer reviewed papers would be of greater long-term benefit to both the individual and the university. This style of thesis is therefore likely to become more common in the future because of this.

The objective of the first paper was to demonstrate the capabilities of FTIR spectroscopy when compared to GC-MS, the predominant analytical technique for the analysis of latent fingermarks. This paper looks at the capabilities and limitations of FTIR spectroscopy through experimentation and comparison with data obtained from GC-MS analysis. Paper 1 identifies previously unreported observations regarding the relationship between functional groups, and presents results, such as intra-variability within latent fingermarks, which confirms data from previous studies. Paper 1 laid the foundations for all subsequent papers in this thesis.

Paper 2 investigates the effect of moderate temperature variations on latent fingermark chemistry. A previously neglected area of research within fingermark analysis, this paper demonstrates the ability of FTIR spectromicroscopy to analyse a latent fingermark as a system, and the impact of moderate temperatures on that system in real time.

Paper 3 again demonstrates the versatility of FTIR spectroscopy by looking at simplified analogue solutions of latent fingermarks. This paper aims to better understand the intermolecular interactions that take place within these simplified solutions and their potential implications for natural fingermark chemistry.

Paper 4 investigates the temporal degradation of exogenous particulate within latent fingermarks, another issue yet to be answered in the literature. This paper focused on spectroscopic imaging to map a fingermark region and identify illicit compounds as they age and interact with the degrading endogenous components.

Chapter	Paper	Objective	Title	Journal	Status
4	1	1, 2, 3, 4	The Chemical Analysis of Latent Fingermarks: Capabilities and Limitations of FTIR Spectroscopy, a GCMS Comparison	-	-
5	2	2	The effect of moderate temperatures on latent fingerprint chemistry	*Applied Spectroscopy*	Applied Spectroscopy, 71 (9) (2017), **2102-2110**
6	3	3	A study of the intermolecular interactions of lipid components from analogue fingermark residues.	*Science and Justice*	Science and Justice, 58 (2) (2018) **121-127**
7	4	4	The temporal degradation of illicit contaminants in latent fingermarks using Fourier transform infrared spectroscopic imaging	*Forensic Science International*	Under review
8, 9			Discussion, conclusions and contribution to knowledge	This section will link the results from the different papers to deliver a coherent body of work	

Table 1: Thesis structure

Each paper contains related literature, methodology, results and discussion of key findings. Although each paper is free standing, in the sense that they each investigate different facets of latent fingermark chemistry, they are all related to an overarching theme of the applications of FTIR spectroscopy for the analysis of latent fingermarks. Thus, the thesis can be viewed as a combined contribution from a collection of the four papers.

CHAPTER 2. INTRODUCTION

Outline

This chapter provides an essential background to the subject area that will be expanded upon in the papers presented in the main body of work. An overview of latent fingermark chemistry and variability is outlined, as well as a summary of the current analytical techniques used for the investigation of fingermarks. Finally, a more detailed review of the use of FTIR spectroscopy for the analysis of latent fingermarks is provided.

Analysis of the unique patterns that make up an individual's fingermarks is arguably the oldest form of forensic investigation on record, with references dating back to China 200 BC. Following the fingerprint revolution of 19th century, the London Metropolitan Police were the first institution to officially use fingermarks for the purposes of forensic investigations in 1901. Today, the use of fingermark ridge patterns to identify individuals remains an invaluable tool for law enforcement agencies and even outperforms DNA in its specificity, no two fingermarks have ever been found alike in the many billions of human and automated computer comparisons conducted around the world, which cannot be said for DNA.

The molecular composition of latent fingermarks, however, is a relatively emerging area of forensic science due to their immense complexity, with numerous factors affecting their chemical behaviour. There are significant challenges, therefore, in utilising fingermark chemistry for forensic science applications. For example, fingermark chemistry exhibits significant intra- (from the same individual), and inter-variability (between individuals), and they are highly susceptible to changes over time [1]. Latent fingermarks are also affected by endocrine factors [2], as well as variations in environmental conditions.

Interest in fingermark chemistry has intensified over the last decade primarily due to advances in analytical instrumentation, but despite this increase in research an in-depth understanding is yet to be achieved [3].

One analytical technique utilised within this field is Fourier Transform Infrared (FTIR) spectroscopy and spectromicroscopy. FTIR analysis is fast, requires no, or minimal sample preparation and, perhaps most importantly from a forensic science perspective, is non-destructive. Yet, although FTIR spectroscopy has many attributes that are appealing to the forensic community, it is yet to become an established tool within forensic investigations.

Gas chromatography-mass spectrometry (GC-MS) remains the gold standard for the chemical analysis of latent fingermarks. GC-MS (or MS derivatives) have been used for the majority of investigations into fingermark composition and aging, providing detailed, quantifiable analysis of the components present within latent fingermarks.

Both FTIR spectroscopy and MS analysis can offer unique insight into the composition of latent fingermarks and could arguably be considered complementary techniques for such analysis. A greater appreciation of the capabilities and limitations of FTIR spectroscopy, however, would allow for a more in-depth understanding of the complex and dynamic nature of latent fingermarks.

This document will demonstrate the applications of FTIR spectromicroscopy as a rapid, non-destructive tool for the analysis of latent fingermark chemistry. The ability of spectral imaging to identify, and spatially map aged illicit substances within latent fingermarks will also be demonstrated, a capability which could be of immediate use to the forensic community.

2.1 The chemical components of latent fingermarks

The chemical composition of latent fingermarks is both intricate and varied. A fingermark is a complex composition of hundreds of compounds secreted from primarily sebaceous and eccrine glands located within the dermis of the skin (figure 1).

Figure 1: Location of eccrine and sebaceous glands within human skin anatomy [Google images ©]

(N.B. Apocrine glands are another class of secretory gland but are typically ignored when considering the chemical make-up of latent fingermarks. This is in part due to their locations on the body, primarily found under the arms and around the genitalia, but also due to their secretions being affected by eccrine and sebaceous gland contamination [4]*)*

Sebaceous deposits found in latent fingermarks are not secreted at the fingertips, however, but deposited onto them through contact with other parts of the body, such as the face and neck, known as grooming behaviour. This means that the concentrations of certain constituents within a fingermark can vary significantly from one individual to the next. To add further complexity latent fingermark chemistry is not static, components undergo decomposition through oxidation and bacterial degradation almost immediately after deposition, known as fingermark aging.

The study of fingermark aging is arguably the dominant area of research within the field. The ability to establish the time since deposition of a latent fingermark, through its state of degradation, would have significant implications for crime

scene investigators when looking to potentially rule a suspect in or out of an investigation. Yet fingermark degradation is still a very active area of research as a reliable model to establish time since deposition is far from being universally accepted.

Various studies, spanning back decades, have looked to establish the contents of fresh latent fingermarks. This in itself has been a challenge given the detection limits of various analytical techniques, although much of the influential work into the composition of latent fingermarks has almost exclusively utilised GC-MS until more recently [1, 4-8]. Mass spectrometry remains the principle technique for the analysis of fingermark composition, and with the addition of soft ionisation techniques, such as Matrix Assisted Laser Desorption/Ionisation (MALDI), MS continues to identify previously unreported components.

Typically, a latent fingermark is comprised of three groups of components, namely sebaceous, eccrine, and squames (skin cells).

2.1.1 Sebaceous secretions

Sebum is an oily, waxy substance secreted by the sebaceous glands, found primarily at the base of hair follicles, its function being to waterproof and lubricate the skin. The sebaceous glands are located throughout the body, except for the palms of the hands and soles of the feet [9]. The density and distribution of these glands are greatest around the face and scalp, where up to 800 glands per cm^3 can be found [4]. Sebaceous glands are fully developed and functioning before birth, most likely due to stimulation by maternal hormone signalling, however, at birth, these glands become mostly inactive due to the withdrawal of this maternal hormonal stimulus [10]. The glands remain inactive until stimulated by particular androgenic hormones, in particular, testosterone and aldosterone, at puberty [11]. Indeed, studies, where testosterone has been administered to children, reported a significant increase in sebum demonstrating that endogenous testosterone is essential for sebum production [12].

Sebum itself is produced by a holocrine mechanism, i.e., sebum-filled cells rupture, disintegrate and empty their contents, via sebaceous ducts, onto the skin surface [13].

As sebum production and secretion is under hormonal control, it varies considerably in composition and consistency, but is generally composed of fatty acids, wax esters, triglycerides, squalene - a steroid precursor, and cholesterol (table 2) [4].

Organic (major)	
Triglycerides	30–40%
Free fatty acids	15–25%
saturated	50%
monounsaturated	48%
polyunsaturated	2%
Wax esters	20–25%
Squalene	10–12%
Cholesterol	1–3%
Cholesterol esters	2–3%

Table 2: A summarised composition of sebaceous secretions [4]

Sebum composition is not only subject to hormonal regulation however, and, although the general composition of sebum is now accepted, various factors such as diet, genetics and activity levels, can influence that composition between individuals making sebum unique from person to person [14].

2.1.1.1 Fatty acids

The amount of free fatty acids in sebum varies considerably, an average estimation being between 15-25% of total sebum composition [3]. These free fatty acids are derived primarily from the hydrolysis of triglycerides and wax esters due to oxidation and bacteriological processes [15]. It has been reported that free fatty acids increase in concentration over the first 15 days post-deposition within a latent fingermark, due to this hydrolysis of glycerides, wax esters and long chain fatty acids [1], but at a particular concentration the pH drops and inhibits the bacterial lipases, thus halting much of their production [13].

Changes in fatty acid concentration within fingermarks demonstrates a fundamental issue with intra-variability, as it has been observed that fatty acid content can change considerably over time within the same individual. One study reported that certain fatty acids from the same donor taken once a week for seven weeks showed significant variations in concentration [16]. The study also showed a significant difference between male and female fatty acid composition, although this has yet to be reliably demonstrated using large sample populations.

2.1.1.2 Wax esters

Wax esters, comprised of a fatty acid esterified with a fatty alcohol (figure 2), contribute approximately 20 - 25% of adult skin surface lipids [4].

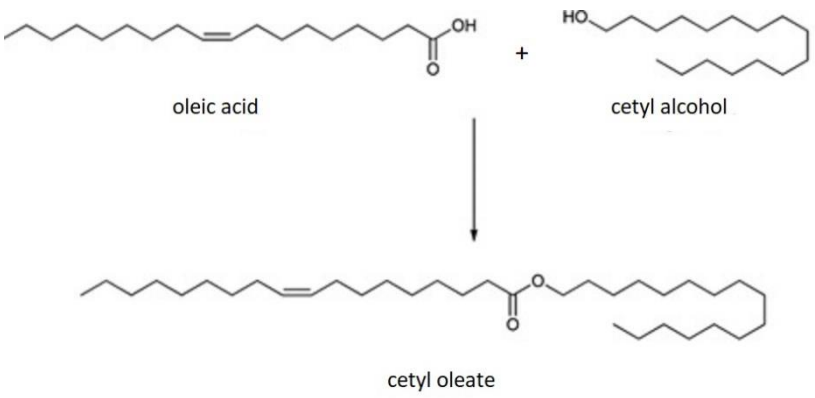


Figure 2: The formation of a wax ester (cetyl oleate) from a fatty acid (oleic acid) and a fatty alcohol (cetyl alcohol)

It is reported that, of the wax ester fatty acids found in adults, ~27% are branched chain fatty acids [4]. This is significant as there are very few branch chain fatty acids found in children's fingermarks, which have a different composition to that of adults and are known to 'disappear' at crime scenes [8, 17-19]. It is reported to be rare to find fully saturated straight chain fatty acid components in wax esters secreted from adults, this is possibly due to their physical state at body temperature, unsaturation or branching making it more likely that the wax ester would be liquid at skin temperature [4].

2.1.1.3 Sterols

Sterols such as cholesterol are secreted from the sebaceous glands and make up between 1-2% of sebum. These compounds are most likely incorporated into sebum from circulatory body fluids (e.g., blood and plasma), and not synthesised within the sebaceous glands themselves [20]. Once on the skin surface bacteriological processes, typically *staphylococci* and *propionibacteria*, esterify cholesterol with (primarily) sebum fatty acids (figure 3) [15]. It has also been reported that sterols and sterol esters are higher in women than men, but, again, this has yet to be sufficiently demonstrated in latent fingermarks to provide a reliable test for sex discrimination. This may, however be due to the decomposition of esters within the fingermarks soon after deposition.

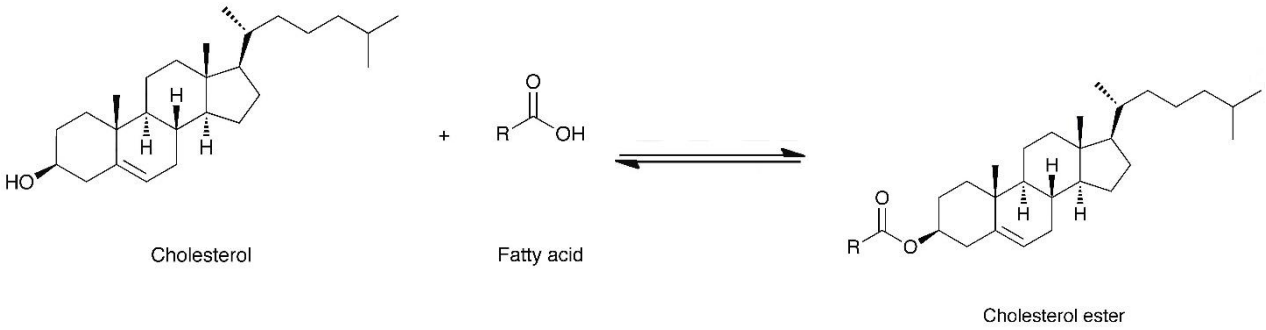


Figure 3: The formation of a cholesterol esters

2.1.1.4 Squalene

Squalene is arguably the single most studied component within sebum regarding fingermark chemistry due to its propensity to rapidly degrade and ease of detection, making it an ideal candidate for modelling fingermark degradation and aging. Squalene, a steroid precursor, makes up approximately 12% of sebum secretions [4], and squalene production has been found to vary with sebaceous gland size. Squalene production has also been shown to vary with age (chapter 2.2), squalene levels are known to be elevated in adolescents for example, and even more so in acne patients, where squalene can constitute to up to 20% of sebum content [21–23]. Variations such as these make squalene a possible candidate for obtaining biometric information from an individual's latent fingermarks.

2.1.2 Eccrine *secretions*

The human skin contains between two and four million eccrine sweat glands distributed throughout the body's surface [24]. In typical individuals, these glands are capable of secreting up to 4 L of fluid an hour. Eccrine glands are fully formed at birth and are active immediately, their highest density being on the soles of the feet and least abundant on the back [24]. Their tubular coiled structure (figure 1) allows the gland to reabsorb essential solutes such as sodium, chloride, bicarbonate, and glucose while allowing water to evaporate from the skin surface. Sweat, secreted from these glands is predominantly made up of approximately 98% water, organic constituents such as proteins, lactic acid and various amino acids, and inorganic salts as well as traces of urea.

2.1.2.1 Inorganic compounds

The presence of inorganic ions within eccrine sweat acts to reduce the vapour pressure of the solution and therefore provides faster evaporation rates for thermoregulation [25]. Although the rate of eccrine sweating is dependent on the amount of water ingested it does not seem to effect sweat composition, and therefore the ion concentrations in sweat remain relatively constant [26]. Sodium is the most abundant ion present in sweat and can seemingly vary immensely from 34 to 266 mEq/L between individuals [24].

2.1.2.2 Amino acids

At least eighteen amino acids have been identified within latent fingermarks, and they are a significant focus of fingermark research. In particular, for the development of visualization techniques, such as ninhydrin which reacts with the amino acids and proteins present within fingermarks. The total amount of amino acids in fingermarks varies from between 0.3 to 2.59 mg/L [27], and various studies have found that serine, glycine, and alanine are the most abundant amino acids within human sweat [28-30]. Amino acid concentrations can, however, vary depending on the sample location on the body [29], and regarding latent fingermarks will vary depending on the amount of grooming behaviour exhibited by the individual.

2.1.2.3 Proteins

The protein content of eccrine secretions can vary from between 15 to 25 mg/dL, and involves over 400 polypeptide components [4]. The majority of the peptides in sweat are at the low end of the molecular weight range, but higher molecular weight proteins have been reported to increase as the rate of sweating increases [31]. The sensitivity of the analytical technique could, however, have a role to play in the identification of these higher molecular weight proteins which are notoriously difficult to detect.

2.1.2.4 Lipids

The detection of lipids in eccrine secretions presents a problem due to the ease of contamination by sebaceous lipids. One study did demonstrate, however, the presence of low concentrations (<0.1 µg/mL) of cholesterol and fatty acids [32].

2.1.2.5 Other constituents

Lactate and urea have been reported at significant levels in sweat (between 10-40 mM) [33], as well trace amounts of creatine, creatinine, and glucose [34]. Various enzymes have also been detected in dissected sweat including acetyl cholinesterase, acid phosphatase and monoamine oxidase [35]. These compounds, however, have little impact on latent fingermark analysis due to their low concentrations.

2.1.3 Squames

The body desquamates up to 40,000 skin cells each day [4] so the number of skin cells within a latent fingermark can vary greatly. Their formation originates within the epidermis (figure 1), the cells move from the underlying basal lamina, known as basal cells, where they are still capable of division, through the prickle and granular cell layers, to the outer *stratum corneum* where they are shed. As these cells transition from one layer to the next the organelles within them disappear, and the cells become flattened scales or squames, filled with densely packed keratin and interconnecting collagen. These outer squames are naturally shed from the *stratum corneum* (horny layer) of the epidermis, and it is these cells that are observable in fingermarks.

2.1.4 Typical contents of a latent fingermark

Table 3 shows a comprehensive (although not exhaustive) list of the components currently identified as present within latent fingermarks, and their associated component group [3-6, 28, 30, 36-38].

Component	Source
Amino acids	
alanine	Eccrine
serine	Eccrine
proline	Eccrine
valine	Eccrine
cysteine	Eccrine
leucine	Eccrine
isoleucine	Eccrine
asparagine	Eccrine
aspartic acid	Eccrine
glutamic acid	Eccrine
glycine	Eccrine
ornithine	Eccrine
threonine	Eccrine
histidine	Eccrine
lycine	Eccrine
phenylalanine	Eccrine
tyrosine	Eccrine
Fatty acids	
decanoic acid	Eccrine/sebaceous
tridecanoic acid	Eccrine/sebaceous
13-aminotridecanoic acid	Eccrine/sebaceous
palmitoleic acid	Eccrine/sebaceous
oleamide	Eccrine/sebaceous
Eicosanoic acid	Eccrine/sebaceous
henicosoanoic acid	Eccrine/sebaceous
docosanoic acid	Eccrine/sebaceous
tetracosanoic acid	Eccrine/sebaceous
hexacosanoic acid	Eccrine/sebaceous
tetradecanoic acid	Eccrine/sebaceous
oleic acid	Eccrine/sebaceous
stearic acid	Eccrine/sebaceous
dodecanoic acid	Eccrine/sebaceous
pentadecanoic acid	Eccrine/sebaceous
glutamic acid	Eccrine/sebaceous
hexadecanoic acid	Eccrine/sebaceous
cis-9-octadecanoic acid	Eccrine/sebaceous
nonadecanoic acid	Eccrine/sebaceous

Peptides	
SSL-25	Eccrine
VPD-42	Eccrine
DCD-1L	Eccrine
LEK-45	Eccrine
Inorganic Ions	
chloride	Eccrine
sodium	Eccrine
potassium	Eccrine
iron	Eccrine
calcium	Eccrine
bicarbonate	Eccrine
sulphate	Eccrine
phosphate	Eccrine
flouride	Eccrine
bromide	Eccrine
iodide	Eccrine
magnesium	Eccrine
zinc	Eccrine
copper	Eccrine
cobalt	Eccrine
lead	Eccrine
manganese	Eccrine
molybdenum	Eccrine
tin	Eccrine
mercury	Eccrine
Others	
cholesterol	Sebaceous
squalene	Sebaceous
urea	Eccrine
glucose	Eccrine
lactate	Eccrine
pyruvate	Eccrine
creatine	Eccrine
creatinine	Eccrine
glycogen	Eccrine
uric acid	Eccrine
glycerides (mono, di, and tri)	Sebaceous
wax esters	Sebaceous
cholesterol esters	Sebaceous
Collagen	Squames
Keratin	Squames

Table 3: Components of adult latent fingermarks

2.2 Variations in latent fingermark chemistry

The composition of latent fingermarks from one individual can vary significantly (intra-variability), as well as from different individuals (inter-variability). As mentioned previously various factors can lead to both intra- and inter-variation in fingermark composition. Age, diet, genetics, personal grooming habits, individual metabolism and androgenic hormone levels in the blood, will all affect sebaceous gland secretions. Eccrine secretions can be influenced by environmental conditions, physical activity, and activity within the autonomic nervous system.

Variations in sebum composition have been shown to change depending on age, (table 4), and, as alluded to previously, the effect of hormones on sebaceous glands has a marked effect on sebum production. The rates of sebum excretion, fatty acid concentrations, the ratio of wax esters to cholesterol, and cholesterol esters are all found to change depending on hormonal influences [39]. In one study the largest change in sebum production occurred between the ages of 12 and 13 in both males and females [40]. Some studies have shown that sebum production continues to increase with age, peaking in the mid-thirties and then begins to decline through middle age [41]. Although this is in contrast to other research which showed no significant changes in sebum composition until old age, most likely due to a reduction in hormonal stimulation of the sebaceous glands [10, 42].

Age	Free Fatty Acids	Triglycerides	Wax Esters	Cholesterol	Cholesterol Esters	Squalene
5 days	1.5	51.9	26.7	2.5	6.1	9.9
1 month–2 years	20.8	38.4	17.6	3.7	10.3	9.4
2–4 years	22.9	49.6	8.0	4.2	8.9	6.2
4–8 years	15.9	45.6	6.9	7.2	14.6	7.7
8–10 years	17.8	47.4	17.8	3.2	5.7	8.3
10–15 years	18.8	42.9	23.6	1.8	4.2	8.4
18–45 years	16.4	41.0	25.0	1.4	2.1	12.0

Source: Ramasastry, P., Downing, D. T., Pochi, P. E., and Strauss, J. S., Chemical composition of human skin surface lipids from birth to puberty, *J. Invest. Dermatol.,* 54(2), 143, 1970. With permission.

Table 4: Changes in sebum concentration with age [4]

There are more recognised detectable differences in fingermark composition between adults and children [8, 18, 19, 43]. The composition of children's fingermarks has been studied to a greater extent, due to their reported tendency to 'disappear' at crime scenes faster than that of adults, making lifting and identification of these fingermarks more difficult. Pre-pubescent children's latent fingermarks have higher concentrations of volatile unesterified free fatty acids (figure 4), while adult's fingermarks contain less volatile fatty acid esters [18] (figure 5). Children's latent fingermarks also have fewer low volatility branched fatty acids [19], and therefore vaporise more quickly. Other studies, however, have suggested that certain children's fingermarks can still be distinguished from adults up to four weeks after deposition [18, 19] suggesting that even aged fingermarks can be classified according to maturity.

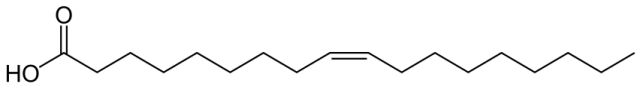


Figure 4: Free fatty acid (Oleic acid) in higher concentrations in children (pre-pubescent)

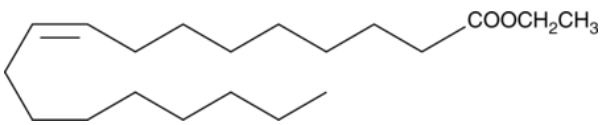


Figure 5: Fatty acid ester (Oleic acid ethyl ester) in higher concentrations in adults

Inter-variation due to gender has been indicated by the amino acid and lipid content of latent fingermarks [7]. The average percentage of five key fatty acids (palmitic acid, palmitoleic acid, oleic acid, myristic acid, pentadecanoic acid) being higher in men than in women; this small study determined, however, that no statistically significant gender variations were detected. It could be surmised that men have higher concentrations of fatty acids in their fingermarks due to higher levels of androgens (testosterone, androstenedione) in the bloodstream, but this has yet to be conclusively proven.

Latent fingermarks not only vary between individuals or groups, but they also exhibit changes over time, some examples of which have been touched on previously. This complex system changes from an "initial composition" state to

an "aged composition" state. The change from initial to aged state is dependent on factors that have been classified into five groups [3]: 1) donor characteristics, 2) deposition conditions, 3) substrate nature, 4) storage conditions, 5) contaminations.

There are some key temporal changes in fingermark chemistry that are consistently observed. The complex and dynamic nature of latent fingermarks means that there is not merely a reduction in quantity of components as they age. Squalene, for example, oxidises quickly (<15 days) to various hydroperoxides, particularly in light conditions, to produce a number of short chain fatty acid by-products. Another example being certain short-chain fatty acids (hexanoic, octanoic, nonanoic acids), as mentioned previously, increase in concentration in the early stages of aging and then decrease back to original levels after 15 days [1]. This area of research is a critical aspect of fingermark chemistry and is investigated in more detail in the papers section.

2.3 Destructive and non-destructive analytical techniques for latent fingermark analysis

2.3.1 Destructive analysis

2.3.1.1 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS has been one of the most utilised analytical techniques for the study of latent fingermarks. The majority of research into fingermark composition, variation, and aging, particularly when the research was in its infancy, was carried out using GC-MS, due to its high sensitivity and specificity.

A separation (GC) and detection (MS) method, GC-MS is ideally suited for the determination of volatile and semi-volatile organic compounds in complex mixtures, such as in fingermarks. GC can separate many volatile and semi-volatile organic components, but not always selectively detect them, whereas MS can detect compounds but not always separate them. To achieve separation, the sample is dissolved in an organic solvent, often dichloromethane (DCM), and, through an injection port is injected into a mobile phase at an elevated temperature. A carrier gas, usually helium, then carries the vapourised sample through the stationary phase, typically a capillary column, at a particular flow rate, 2.40 mL/min for example. The analyte in the mobile phase interacts with the stationary phase, each component of that analyte interacting at a different rate, thus separation is achieved. Those compounds that interact the fastest, for example, will exit, or elute, from the column first. As the carrier gas moves the analyte through the column the temperature is increased in steps, or 'ramped', typically up to 300^{o}C, this increases the degree of separation.

As the analyte components elute from the GC column, they enter the mass spectrometer and are bombarded by a stream of electrons, causing them to ionise and fragment. The charged ions are then focused around a path by a magnetic field through a slit into the detector which, using the radius of that curved path, can identify the mass and relative abundance of that fragment, and therefore provides a unique fingerprint for that compound. Once the entire sample is analysed the resulting mass spectrum (figure 6) can be compared to a library to identify the compounds present.

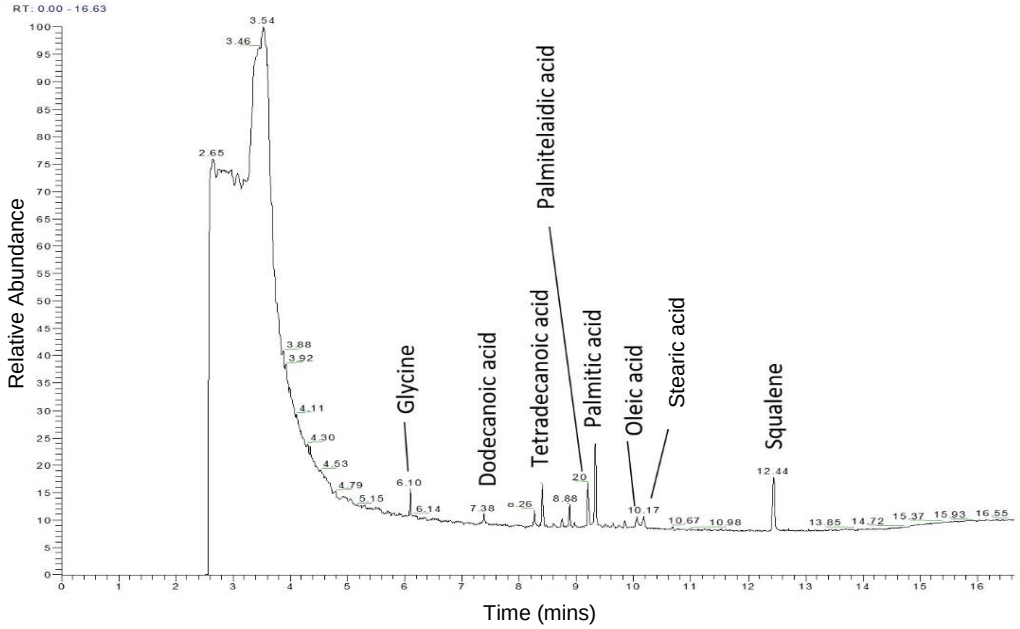


Figure 6: Typical chromatogram of a latent fingermark

Seminal work investigating latent fingermarks to identify the key components, such as sebaceous and eccrine content [5, 6, 44, 45], changes in sebum composition over time [1, 4, 6], and differences in children's and adults' fingermarks [19], were all investigated using GC-MS analysis, as well as investigating the chemical composition of fingermarks for gender determination [7]. GC-MS enables the quantification of specific fatty acids and lipids found in low concentrations within fingermarks (figure 6), and the high sensitivity allows for detailed analysis of intra-variability, changes in fingermark chemistry over time, or under different environmental conditions.

One of the main shortcomings of this technique, however, is that it is limited in its ability to analyse the entire contents of a fingermark. The technique depends on the vapour pressure of a compound for detection, making large, low volatility molecules difficult to detect. Various studies have shown that, although GC-MS can consistently detect a broad range of lipids and amino acids, larger molecules (>C28) such as long chain lipids and wax esters, cholesterol and myristyl myristoleate for example, are less consistently detected [1, 5, 6, 17].

These larger compounds are sometimes only identifiable when at abnormally high levels, such as in individuals with hyperlipidemia (high blood cholesterol).

This inconsistency in detection is due to a combination of factors; namely volatility, the variability innate of latent fingermarks, and the low concentrations of many of these compounds within eccrine and sebum deposits generally [4]. GC-MS is also unable to detect much of the higher molecular weight protein content within latent fingermarks due to their large size and low vapour pressure. Although protein content in fingermarks is not typically used for aging or variation studies, there is some evidence that the quantity of protein in a fingermark can be used for sex discrimination and possibly to differentiate children's fingermarks from that of adults [55].

The requirement for sample destruction and the time-consuming nature of GC-MS analysis has spurred a drive to investigate other more rapid, non-destructive analytical techniques in recent years. GC-MS is likely to remain a critical analytical tool however for the analysis of latent fingermarks by forensic laboratories due to its established reliability, and due to operational requirements (primarily for the detection of illicit substances). Even as a research tool, the quantitative specificity and sensitivity of GC-MS for the detection of specific compounds (such as fatty acids, squalene, and triglycerides) which are essential for potentially aging an individual, or the fingermark, and its ability to detect exogenous particulate within latent fingermarks, is unique. It is therefore likely that any new technique will serve as an orthogonal technology or as an on-scene presumptive test, to work alongside or more likely prior to laboratory-based GC-MS analysis.

2.3.1.2 Surface assisted laser desorption/ionisation-time of flight-mass spectrometry (SALDI-TOF-MS)

SALDI-TOF-MS is a variation of mass spectrometry (MS) originally designed for the separation of large organic molecules, and as such has been used in a number of latent fingermark studies. This soft ionisation technique (i.e. very little fragmentation of the sample molecules) has the advantage of reducing sample preparation compared to that of conventional GC-MS. The time saving of the preparation stage, however, is sometimes lost in an extra incubation and sample washing stage, which is not required in GC-MS analysis. Typically, latent fingermark samples analysed via SALDI-MS require dusting with a specific dusting powder, such as magnetic silica nanoparticles or magnetic dusting powder, that not only enables location of the fingermark but then acts as a signal enhancing agent for the technique. SALDI-TOF-MS has been shown to be highly effective in the separation of both biological material, nitro-organic and peroxide explosives, as well narcotics [46, 48, 49]. Modifications to the process using magnetisable solid phase extraction nanoparticles that generate enhanced signal intensity have shown capable of detecting certain fingermark constituents such as trace amounts of amino acids, fatty acids and squalene [50]. The procedure is cost effective and quick, with much-reduced sample preparation, background interference, however, can swamp any characteristic peaks and it is currently unclear why only certain amino acids are detected.

2.3.1.3 Matrix Assisted Laser Desorption/Ionisation-time of flight-mass spectrometry (MALDI-TOF-MS)

MALDI-MS is again a variation of fundamental MS, and is often used in the study of macro-molecules such as proteins, due to its sensitivity, lack of fragmentation, and relative speed of preparation. Peak intensities, however, can be significantly diminished when compared to SALDI-TOF-MS, and as with SALDI-TOF-MS, a critical ‘make-or-break' step is the successful lifting of the fingermark from the surface. Preparation for MALDI-TOF-MS analysis requires the sample placed on a conductive surface and coated with a matrix, α-cyano-4-hydroxycinnamic acid (CHCA) for example. This matrix absorbs a specific laser wavelength and therefore ionises the analyte. The sample is then analysed using the mass spectrometer, typically under pressure. The use of MALDI-TOF-MS in the analysis of latent fingermarks has revealed the presence of a broad range of lipids, fatty acids and previously unknown peptides [36]. MALDI-TOF-MS has established the distribution of endogenous lipids in groomed and ungroomed fingermarks, and demonstrated spatial maps showing ridge patterns, allowing for specific compound identification and their location within the fingermark [51-53]. MALDI-TOF-MS has also been used for the detection and mapping of narcotic particulates present within latent fingermarks [49]. This analytical technique is currently receiving significant attention as the quantity of retrievable information from a suspect’s fingermarks using this method is high [54]. MALDI-TOF-MS has shown promise in identifying biometric information, particularly the sex of an individual [55]. Combining MALDI-TOF-MS and multivariate modelling enabled fast analysis of latent fingermarks to differentiate sex through specific peptide and small protein composition with a validated prediction accuracy range from 67.5% to 85%. It is believed that with refinement of the sample preparation methodology this accuracy could be improved. MALDI-MS imaging and profiling (MALDI-MSI/P) has recently been adopted by West Yorkshire Police, UK, for existing police casework, and may be admissible evidence in UK courts ‘within months' [56].

2.3.1.4 Electrospray ionisation mass spectrometry (ESI-MS)

ESI-MS is an alternative MS technique less frequently used for latent fingermark analysis. ESI, another soft ionisation technique, is better suited to polar molecules; many of which occur in the form of the lipid oxidation products prevalent when fingermarks age. ESI-MS is limited in its ability to analysis anything more than fatty acids and some lipids present in latent fingermarks, though because of the lack of fragmentation, it can yield fine detail of the oxidation products of these compounds. For example, the use of ESI-MS effectively demonstrated the numerous oxidation products of squalene in aging fingermarks [57]. This technique highlighted the impact of UV light on squalene degradation and its oxidation products, which is consistent with previous findings involving the rapid depletion of squalene in latent fingermarks in daylight [3, 5]. The use of this soft ionisation technique showed that many of the squalene (and other fatty acid) oxidation products are short-lived, and these intermediates are then rapidly oxidised again to more stable compounds such as hydroperoxides. ESI-MS analysis has demonstrated the dynamic complexity and chemical variation that occurs within latent fingermarks as they degrade and may play a future role in developing an accurate model for fingermark aging.

2.3.2 Non-destructive analysis

Mass spectrometry has played a pivotal role in the analysis of latent fingermarks for over fifty years and remains the most commonly utilised technique within the field. The major drawback of any form of MS technique, however, is the preparation and analysis time, and most importantly the destruction of the sample, preventing further forensic analysis. During the last decade, there has been a concerted effort, driven by the requirements of the policing and security communities, to develop a method of rapidly and non-destructively analysing latent fingermarks. The ultimate aim being to analyse latent fingermarks in the field, ideally *in situ* at the crime scene, or perhaps more likely in a mobile forensic laboratory, to establish biometric information on the fingermark donor and/or to identify any illicit compounds within the fingermark. Recent developments in the accuracy and specificity of spectroscopic techniques such as Raman and in particular FTIR spectroscopy have demonstrated the potential to meet these requirements.

2.3.2.1 Raman Spectroscopy

Raman spectroscopy has been well established as a forensic tool since the beginning of the century. It has been used for drug and explosive identification, currency verification and for the verification and aging of works of art [58-60]. Raman spectroscopy has also been utilised in the biomedical industry to identify specific protein and amino acid markers, for example in diabetic patients [61].

Raman spectroscopy is often considered a complementary technique to FTIR due to their mutual exclusion of molecular symmetries from a molecule of interest. That is to say, a vibrational mode that is inactive to IR will be active to Raman (figure 8) and vice versa. Raman spectroscopy occurs when inelastic scattered photons are collected and their energy differences translated into a wavenumber indicative of the vibrating bonds within a molecule. Raman spectroscopy, however, is not so commonly used in the analysis of latent fingermarks; despite evidence that this spectroscopic technique can yield distinct benefits. Fingermarks in moist or humid conditions, for example, or of eccrine specific samples, are better suited to Raman spectroscopy. Surface-enhanced Raman

spectroscopy (SERS) is a technique where the sample is placed onto a nanoscale roughened metal surface which provides enhanced intensity by reducing Raman scattering. This reduced scattering significantly decreases the background interference giving a much-enhanced signal that is not affected by moisture as with FTIR. SERS has been shown to be capable of producing certain chemical images of latent fingermarks, and identifying a broad range of amino acids and other eccrine constituents [58]. Raman spectroscopy has also been used in a limited capacity to detect illicit substances, primarily narcotics [60, 62] and certain trace explosives in latent fingermarks [63].

One reason Raman spectroscopy has been of limited use for the analysis of latent fingermarks is because Raman is less specific in identifying sebaceous secretions when compared to FTIR spectroscopy. As mentioned previously, sebaceous compounds are critical in the aging of fingermarks, and current research into the use of latent fingermarks as biomarkers is almost exclusively concerned with the lipids predominantly produced by sebaceous secretions. Another issue with Raman spectroscopy is the limited ability to chemically image fingermark samples; yet this has been established in (particularly mid-range) FTIR analysis for a number of years.

2.3.2.2 Fourier Transform Infrared spectroscopy (FTIR)

Fourier Transform Infrared spectroscopy dominates the non-destructive analytical tools utilised for the analysis of latent fingermarks. Although Raman and FTIR spectroscopy share many benefits for fingermark analysis, they also have differing strengths. As mentioned previously, FTIR yields better spectra for organic molecules and is therefore generally more suitable for the analysis for latent fingermarks. Initial studies into latent fingermark chemistry using FTIR spectroscopy identified the key components; namely sebaceous and eccrine deposits, as shown in figure 7 [64-66]. Each peak indicating a key functional group that corresponds to the compounds present within a latent fingermark (detailed in section 2.1).

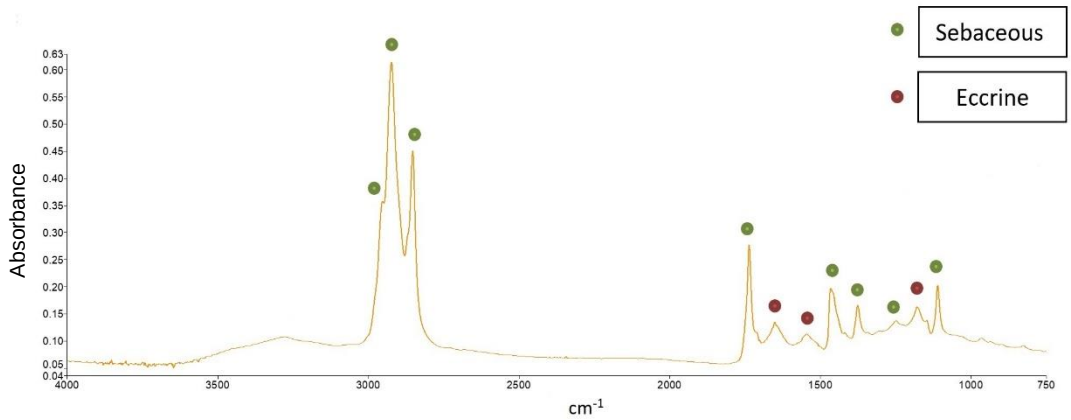


Figure 7: Typical FTIR spectrum of a latent fingermark

This ability to identify the functional groups present within a latent fingermark is a significant advantage of FTIR spectroscopy, a weakness, however, is that it does not permit the differentiation of one organic compound from that of another with a similar molecular structure. For example, it is impossible to distinguish between the fatty acids palmitic acid ($CH_3(CH_2)_{14}COOH$) and stearic acid ($CH_3(CH_2)_{16}COOH$) due to their structural similarity and therefore similar IR absorbance. In the spectrum in figure 7 these two fatty acids, along with many others, would be contained within the sebaceous bands (green peaks).

The use of FTIR spectroscopy to analyse groups of compounds with similar structures, such as fatty acids, lipids, and amides, has however proven useful when comparing variability between latent fingermarks. The previously

mentioned anecdotal evidence from police and scenes of crime officers that children's fingermarks 'disappear' after a short period of time [8] was corroborated by observed chemical differences using both GC-MS [18,19] and FTIR spectroscopy [43]. Using latent fingermarks as a biometric gauge for aging an individual has also been demonstrated using FTIR spectroscopy [67], and with greater granularity than merely child or adult. Using specific regions of the FTIR spectra (1190-1260cm^{-1} & 2828-2970 cm^{-1}) initial studies indicate that the study group ranging from 4 to 64 years of age could be divided into four populations: 4-5, 11-14, 18-26 and 29-64 years old. This is primarily due to the changes in concentrations of free fatty acids, in higher concentrations in children, and esterified fatty acids, in higher concentrations after puberty. The suggested benefit of this research is that this technique could be used to provide an age category for an individual when fingermarks found at a crime scene during a forensic investigation provide no matches from a criminal database.

Despite the inability of FTIR spectroscopy to provide the specificity of MS instruments, the ability to identify component groups within a latent fingermark non-destructively suggests the potential for a new capability of on-scene forensic analysis.

FTIR spectromicroscopy has been shown capable of analysing latent fingermarks directly from evidence such as bank notes, rubbish bags, drinks containers, duct tape and copier paper [65] without damaging the fingermark (if it is known where to locate the mark). This is of particular relevance for forensic analysis because it allows the fingermark to be unaltered by developing techniques which risk corrupting any associated forensic evidence such as exogenous particulates or fibres.

A major issue with analysing undeveloped fingermarks is that these marks are rarely found at a crime scene. It is likely that the fingermark will only be identified via a development technique such as conventional development powder, magnetic powders, fluorescent dusting, cyanoacrylate (superglue) fuming and so on. To date, there has been very limited research into the effects of fingermark development techniques on FTIR analysis, yet it is essential that we understand

the impact of these techniques if FTIR spectroscopy is to become a practical analytical tool for the forensic analysis of latent fingermarks.

At present, there have been various studies into the detection of trace quantities of illicit particulate within latent fingermarks using FTIR spectral imaging [47] and synchrotron radiation-based FTIR (SR-FTIR) [68], which have relevance for the forensic, policing and intelligence communities. SR-FTIR allows for high spatial and spectral resolution and reduced background interference, and therefore provides more detailed spectra. This technique has been shown capable of detecting pharmaceutical (aspirin) and explosive (RDX, TNT, PETN) contaminants within latent fingermarks [68] as well mixtures of contaminants (aspirin and sugar). This study demonstrated the possibilities of using FTIR to detect contaminants within latent fingermarks, as well as showing that SR-FTIR can analyse lifted fingermarks which would contain fewer contaminants than the *in situ* originals. SR-FTIR analysis is however not practical for standard forensic laboratory analysis of fingermarks, and, in the referenced study, the whereabouts of the fingermarks were known prior to lifting. Therefore, no fingermark developing techniques were used, which is unrealistic when compared to a genuine forensic investigation. More 'real world' studies for analysing latent fingermarks have been conducted by agencies such as the FBI and the Singapore Police Force [69]. In these studies, contaminated fingermarks were first developed with standard dusting powders, lifted using specific lifting tapes (lifters) and then analysed using an SR light source. These studies confirmed that dusting powder adds a level of complexity in locating and analysing any illicit particles in the visual mode but identifying any explosive particulate via its corresponding spectrum is possible. Certain lifting tapes are not suitable for direct FTIR analysis due to their non-transparency, and whichever lifter is used, its composition may have implications for the identification of illicit substances.

Attenuated total reflectance (ATR)-FTIR is a modification of FTIR analysis which uses an optically dense crystal for fast analysis of solids and liquid mixtures with no preparation required. This technique has been used to detect a limited variety of explosive particulate (TNT, TNB, AN) in latent fingermarks by placing the ATR

crystal directly onto an explosive particle within the fingermark residue [70]. This study demonstrated that detection of illicit substances using conventional FTIR techniques is possible, but fingermarks were laid down on an ideal, IR transparent surface (stainless steel) for analysis, were not lifted or dusted, and as with the previously mentioned study, the contaminants were known prior to analysis.

2.4 The principles of Fourier Transform Infrared spectroscopy

2.4.1 Overview

Fourier Transform Infrared spectroscopy is a non-destructive analytical spectroscopic technique used to obtain analytical data from a sample. FTIR spectroscopy uses infrared (IR) radiation which passes through a sample and is absorbed at specific wavelengths dependent on the functional groups within the molecular structure of the analyte. A sample will produce a unique spectrum depending on the compounds under analysis, therefore spectral data obtained from IR analysis is an extremely effective way of analysing functional groups. The absorption of this radiation results in an increase of energy proportional to the wavelengths absorbed from a ground vibrational state to an excited state. For a sample molecule to absorb IR the vibrations and rotations (vibrational modes) within the molecular bonds must cause a net change in the dipole moment (the distribution of positive and negative charges within the molecule). The alternating electrical field within the IR radiation interacts with the fluctuations in the dipole moment of the molecule. If the frequency of the IR radiation matches the vibrational frequency of the molecule, then radiation will be absorbed. As an example, the vibrational modes of carbon dioxide (CO_2) can be both IR active and inactive; as only some of the vibrational modes result in a change in the dipole moment (figure 8).

A molecule with *n* atoms has a definite number of vibrational modes that can occur. The number of vibrational modes is dependent on the shape of the molecule. The number of vibrational modes for a linear molecule, such as CO_2, is $3n$-5 (where n is the number of atoms), for a non-linear molecule, such as H_2O, the vibrational mode is $3n$-6. Taking the example of CO_2, a simple linear triatomic molecule (O=C=O), has 3(3)-5 = 4 vibrational modes, while H_2O, a non-linear molecule has 3(3)-6= 3 vibrational modes. Large organic molecules found in fingermarks, such as lipids and proteins are typically non-linear. The fatty acid stearic acid, for example, has the molecular structure $C_{18}H_{36}O_2$, so has 3(56)-6 = 162 vibrational modes.

As mentioned previously, for a molecule to be IR active there must be a net change in the dipole moment, and this requires specific vibrational modes known as asymmetric stretching or bending. These are the only modes that generate a change in the dipole moment, as shown in figure 8. Symmetric stretching is IR inactive because although there is a change in bond length, the overall shape of the molecule remains the same and therefore there is no change in the dipole moment.

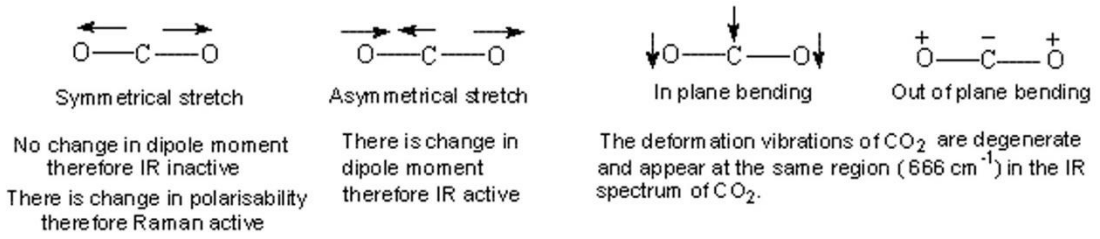


Figure 8: "Ball and spring model" - requirements for a molecule to be IR active

Only the asymmetrical stretching of CO_2 therefore, produces a change in the dipole moment so is IR active. Of the four vibrational modes of CO_2 (3(3)-5=4) only two are seen in an IR spectrum at 2350 cm^{-1} and 666 cm^{-1}, as shown in figure 9.

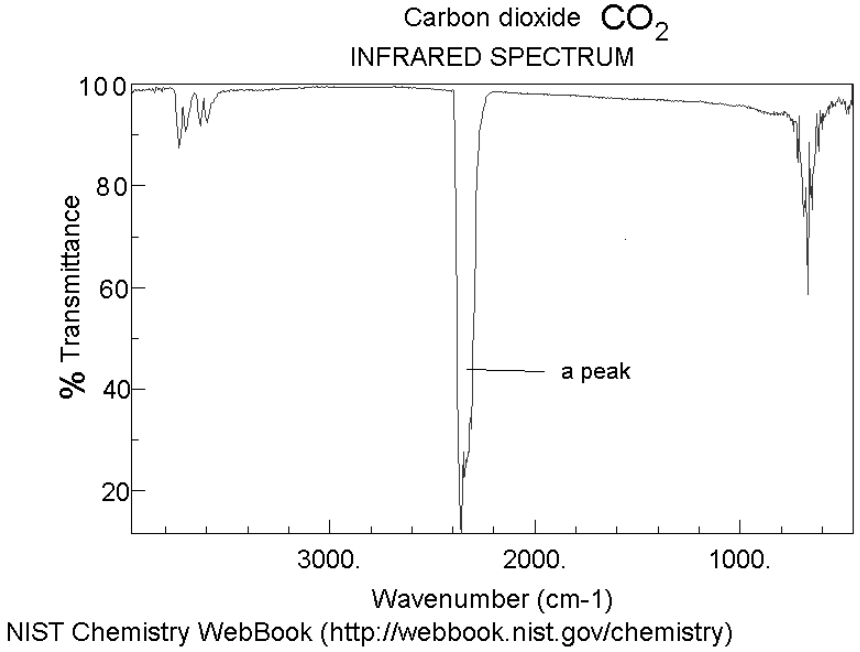


Figure 9: IR spectrum of carbon dioxide

2.4.2 Mid-range IR and fundamental vibrations

Mid-range IR, as used in this study, is the spectral range from 4,000 to 400 cm^{-1}. Mid-range IR is typically used for the analysis of organic materials because various organic functional groups (C-H, C-O, C=O, C-N) show strong absorbance in this region, particularly the fingerprint region from ~1500 to 500 cm^{-1}. Specific functional groups generate unique group frequencies (vibrations that are associated with a specific functional group) which can then be used for analysis.

The ball and spring model, as demonstrated in figure 8, is not entirely sufficient to explain organic molecular excitation, and hence the suitability of mid-range IR. Typically, the quantum mechanical formulism is used to describe discrete energy levels:

$$E_n = h \cdot v \cdot \left(v + \frac{1}{2}\right)$$

Where v is the vibrational number or energy level. According to this selection rule, only transitions are allowed with $\Delta v = \pm 1$. This is because, for most vibrations of organic compounds the ground state, $v = 0$, is occupied under ambient temperatures, the observed IR absorption bands of organic molecules in mid-range IR are caused by the transition $v = 0 \rightarrow v = 1$, known as a *fundamental vibration* (figure 10). As figure 10 shows, this leads to a change from a harmonic oscillator to the anharmonic oscillator model. The result being an asymmetric potential curve, the right part of which converges with the bond dissociation energy.

In Near-infrared (NIR), a growing field within IR spectroscopy and being used more for the analysis of organic materials, the transitions are principally known as *overtones*. Overtones are due to a shift in the selection rule from the harmonic oscillator, with $v = \pm 2, \pm 3$ ..., and are the whole number multiples of the fundamental vibrations (figure 10). Of particular relevance to organic molecules, containing more than two atoms, *combination modes* can also be observed, particularly in NIR. Combination modes refer to the simultaneous excitation of two vibrational modes at one frequency, leading to additional peaks on an IR spectra and can cause complications in analysis.

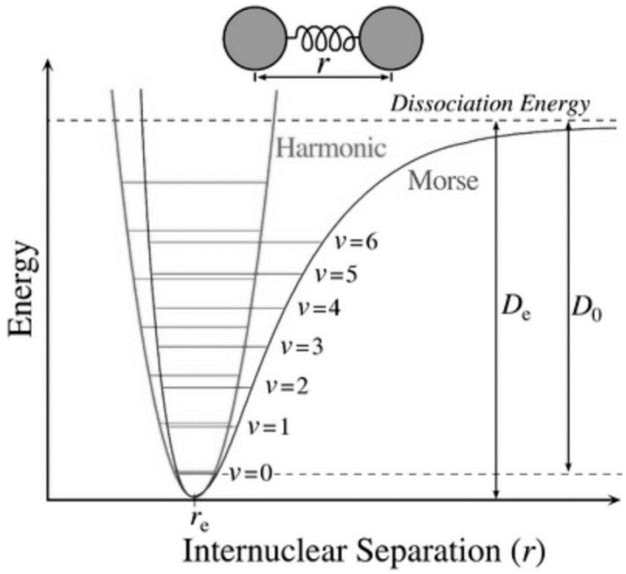


Figure 10: Potential energy curve of an anharmonic oscillator

2.4.3 FTIR Quantitative analysis

The majority of uses for FTIR spectroscopy are in qualitative analysis, using the peaks to identify the presence of specific molecules or functional groups. FTIR can however also be used quantitatively, although the technique is not as accurate as other analytical techniques such as GC-MS.

Samples are quantified using the principle of Beer's Law:

$$A = \log\frac{I0}{I} = eLc \quad \text{or} \quad A = eLc$$

Where:

A is the absorbance of the sample

e is the molar absorptivity (L mol^{-1} cm^{-1})

L is the path length of the sample (cm)

c is the concentration of the sample (mol L^{-1})

Beer's Law tells us that absorbance depends on total quantity of the absorbing sample in the beam path. When absorbance is plotted against concentration, a linear relationship is seen demonstrating that there is a direct relationship between absorbance and quantity. This law does have its limitations however as it does not apply to high concentration samples, and in establishing the concentration, the path length will be required so is an issue for samples of an unknown quantity. The requirements for quantification are not always possible especially in a forensic context where samples will often be unknown and unmodifiable.

2.5 FTIR spectra of latent fingermarks

The FTIR spectra from the three component groups of a latent fingermark are detailed below (NB all spectra and chemical maps were obtained by the researcher):

2.5.1 Sebum

An FTIR spectrum of sebum (figure 11) contains the characteristic lipid peaks that dominate the spectrum of a latent fingermark, primarily the antisymmetric and symmetric C-H stretch of CH_2 groups, from 2920 – 2854 cm^{-1}, and the peaks at 1740 and 1180 cm^{-1} indicative of the C=O and C-O vibrations from carbonyl esters.

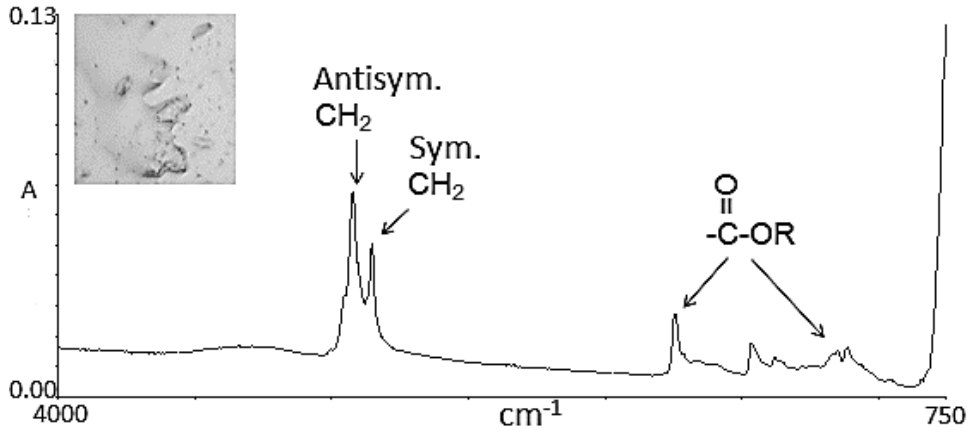


Figure 11: IR spectrum of sebum deposit, note the antisymmetric and symmetric C-H stretch of CH_2 groups between 2920 - 2854 cm^{-1}

2.5.2 Sweat

Eccrine sweat (figure 12) is predominantly comprised of water, inorganic salts, amino acids, lactic acid and traces of urea. Organic acids from sweat can be identified by the carboxylic acid peak between 1780 -1710 cm^{-1}, the broad hydroxyl peak at 3300 cm^{-1} is predominantly water (N.B. a far smaller peak is also present at 3300 cm^{-1} in the presence of protein due to the hydroxyl group).

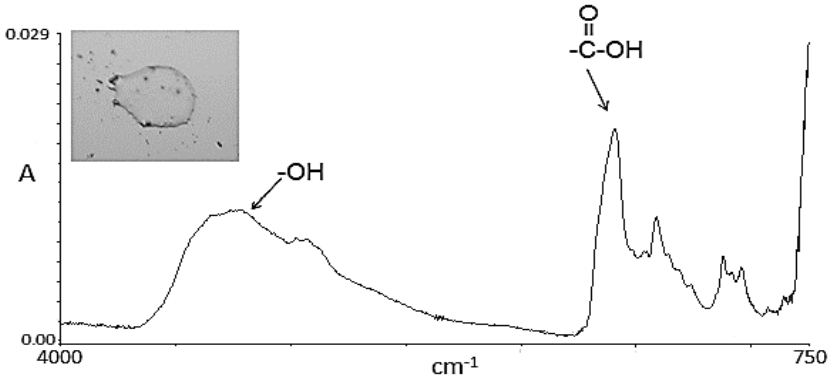


Figure 12: IR spectrum of sweat deposit, note the large carboxylic acid peak between 1780-1710 cm^{-1}

2.5.3 Peptides and proteins

It is possible to identify the amide backbone of peptides and proteins within fingermarks. Specifically, the amide I band between 1700 - 1600 cm^{-1} from the C=O stretching of the amide bond, and the amide II band at 1580 - 1480 cm^{-1} derived from both the N-H bending and C-N stretching within the amide bond (figure 13).

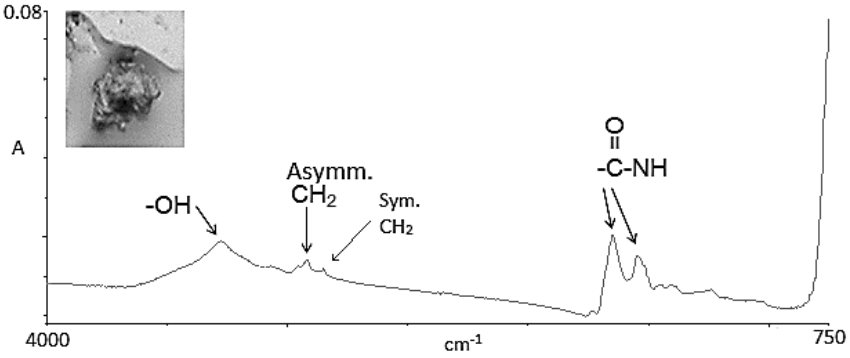


Figure 13: IR spectrum of a skin cell, note the amide I and amide II peaks between 1700 – 1600 cm^{-1} and 1580 – 1480 cm^{-1} respectively.

The spectra shown in figures 11 - 13 are however unrealistic when compared to latent fingermarks found *in vivo*, such as at a crime scene. Fingermarks found in these environments are usually a combination of all three components.

Figures 14 shows a typical FTIR spectrum of a latent fingermark analysed in less contrived circumstances and is more consistent with an individual's natural grooming behaviour. The spectrum in figure 14 shows the dominant C-H stretch peaks at 2920 - 2854 cm^{-1}, the C=O stretch at 1740 cm^{-1}, identifying the presence of ester groups, as well as the amide I and II peaks at 1700 - 1600 cm^{-1} and 1580 – 1480 cm^{-1} respectively. This would indicate that this sample had significant concentrations of lipids and peptide bonds present.

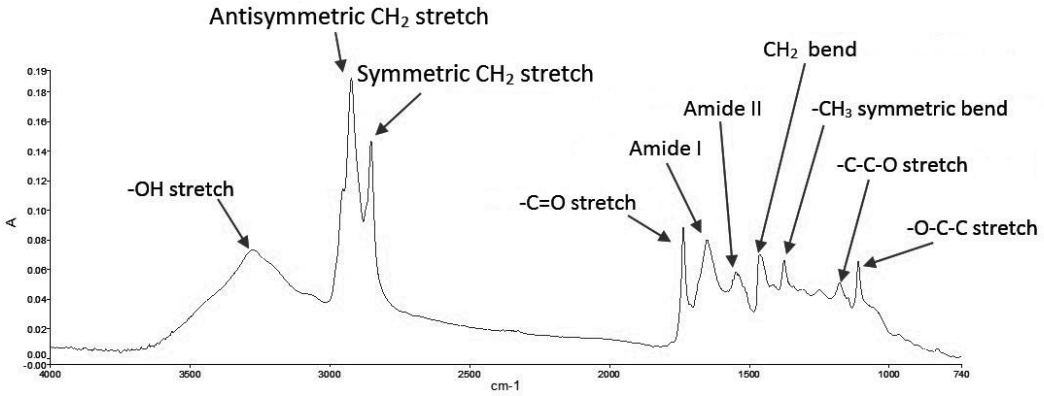


Figure 14: Typical natural fingermark containing sebum, sweat, and protein

Certain key molecular components within latent fingermarks are essential for aging a fingermark with regard to time since deposition, and aging the donor. These components can generally be identified using FTIR analysis. Peaks that are particularly indicative of these key components are the C-O single bond of esters at 1190 - 1260 cm^{-1}, the CH bending and C=O stretching from 1440 -1780 cm^{-1}, and the C=C stretch, again from 1440 -1780 cm^{-1} [67]. It is not, however, possible to specifically identify individual components such as squalene due to the overlap of their spectral bands with that of other sebaceous materials [64].

2.6 Chemical mapping

Because FTIR spectra identify the functional groups of molecules present within a sample, it is possible to generate images that show the distribution of these functional groups using spectral or chemical mapping (chemimaps) based on their absorbance. These images demonstrate the relative abundance and distribution of particular compounds within a fingermark, be they endogenous or exogenous. For example, from a light microscope image (15a), it is possible to produce a chemimap that shows the average absorbance of a latent fingermark (15b), or to isolate a specific functional group such as the carboxylic acid bands at 3000 - 2500 cm^{-1} and 1780-1710 cm^{-1}. The chemimap will show the locations and abundance of this functional group within the fingermark (figure 15c).

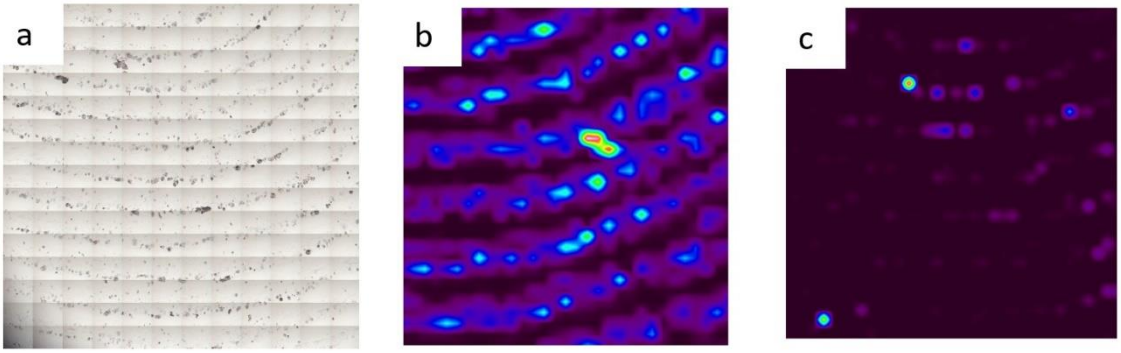


Figure 15: A fingermark image from a light microscope (a), and the corresponding chemimaps showing the average absorbance of that latent fingermark (b), and the distribution of carboxylic acid groups (c).

Figure 15b demonstrates that chemimaps can not only identify the abundance of particular compounds within a fingermark but can also show the ridge patterns. These ridge patterns could, if the entire fingermark were scanned, be used for identification purposes in much the same way as conventional fingermark patterns.

Analysis of the chemical components within latent fingermarks has been undertaken for a number of years, and as the selectivity and sensitivity of analytical techniques has improved our understanding of fingermark composition has developed. An in-depth understanding of the degradation mechanisms that take place within a fingermark post-deposition is yet to be achieved, however. A deeper understanding of the various interactions that take place, the intermediary and end products that develop and in what conditions, would not only advance our knowledge of fingermark chemistry but would be of great value to the law enforcement and forensic community. Such detailed understanding could potentially allow for a multitude of information to be gained from the chemistry of a fingermark. Information such an age range, gender, time since deposition, and the identification of illicit substances.

This thesis, through the presentation of four papers, aims to advance our understanding of latent fingermark degradation by investigating various mechanisms that may affect fingermark chemistry. Aspects of the fingermark degradation process, not previously studied, will be analysed using FTIR microscopy, including the effect of certain environmental factors, the importance of intermolecular interactions on chemical degradation, and the effect of fingermark degradation on illicit contaminants. The results of this body of work will not only aim to advance our academic understanding of fingermark degradation chemistry but provide information that could be of use to the forensic community regarding the behaviour of fingermarks post-deposition, the effect of the environment on the development of fingermarks, and the ability to detect illicit substances in aged fingermarks.

2.7 References

1. N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells, Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface. *Forensic Sci. Int.* 154 (2–3) (2005) 224–239.

2. F.J. Ebling, Steriod hormones and sebaceous secretions, in: Advances in steriods, 2nd ed. London: Academic press*;* 1970. 1-11.

3. A. Girod, R. Ramotowski, C. Weyermann, Composition of fingermark residue: A qualitative and quantitative review. *Forensic Sci. Int.* 223 (1–3) (2012) 10–24.

4. R. Ramotowski, Composition of Latent Print Residues, in: Advanced in Fingerprint Technology, 2nd ed. London: CRC Press; 2001. 63-104.

5. R.S. Croxton, M.G. Baron, D. Butler, T. Kent, V.G. Sears, Variation in amino acid and lipid composition of latent fingerprints. Forensic Sci. Int. 199 (2010). 93–102.

6. G.M. Mong, C.E. Petersen, T.R.W. Clauss, Advanced Fingerprint Analysis Project - Fingerprint Constituents. Vol PNNL-13019. Pacific Northwest National Laboratory: U.S. Department of Energy, 1999.

7. K.G. Asano, C.K. Bayne, K.M. Horsman, M.V. Buchanan, Chemical composition of fingerprints for gender determination. *J. Forensic Sci.* 47 (4) (2002) 805–807.

8. D. Noble, Vanished into thin air. The search for children's fingerprints. *Anal. Chem.* 67 (13) (1995) 435A–438A.

9. R.S. Greene, D.T. Downing, P.E.Pochi, J.S. Strauss, Anatomical Variation in the Amount and Composition of Human Skin Surface Lipid. *J. Investig. Dermatol*. 54 (3) (1970) 240–247.

10. P.E. Pochi, J.S. Strauss, Endocrinologic control of the development and activity of the human sebaceous gland. *J. Inv. Derm.* 62 (3) (1974)191–201.

11. J. Ebling, Hormonal Control and Methods of Measuring Sebaceous Gland Activity. *J. Inv. Derm.* 62(3) (1974) 161-171.

12. J.S. Strauss, P.E. Pochi, The Human Sebaceaous Gland: Its Regulation by Steroidal Hormones and its use as an and Organ for Assaying Androgenicity in Vivo. *Recent progress in hormone research*. 19 (1963) 385 - 444.

13. T. Nikkari, Comparative Chemistry of Sebum. *J. Inv. Derm.* 62 (3) (1974) 257–267.

14. R. Mykytowycz, B.S. Goodrich, Skin Glands as Organs of Communication in Mammals. *J. Inv. Derm.* 62 (3) (1974) 124-131.

15. R. Marples, A. Kligman, L. Lantis, D. Downing. The Role of the Aerobic Microflora in the Genesis of Fatty Acids in Human Surface Lipids. *J. Inv. Derm*. 55 (3) (1970) 173-178.

16. L. Boniforti, S. Passi, F. Caprilli, F. Nazzaro Porro, Skin surface lipids. Identification and determination by thin-layer chromatography and gas-liquid chromatography. *Clinica Chimica Acta*. 47 (2) (1973) 223-231.

17. C. Weyermann, C. Roux, C. Champod, Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis. *J. Forensic Sci.* 56 (1) (2011) 102-108.

18. K.M. Antoine, S. Mortazavi, L.M. Miller, A.D. Miller, Chemical differences are observed in children's versus adults' latent fingerprints as a function of time. *J forensic sci.* 55 (2) (2010) 513-518.

19. M.V. Buchanan, K.G. Asano, A. Bohanan, Chemical Characterization of fingerprints from adults and children. *International Society of Optical Engineering.* 2941 (1996) 89-95.

20. N, Nicolaides, H.C. Fu, M.N.A. Ansari,G.R. Rice, The fatty acids of wax esters and sterol esters from vernix caseosa and from human skin surface lipid. *Lipids*. 7 (8) (1972) 506-517.

21. K.M. Nordstrom, J.N. Labows, K.J. McGinley, J.J. Leyden, Characterization of Wax Esters, Triglycerides, and Free Fatty Acids of Follicular Casts. *J. Inv. Derm.* 86 (6) (1986) 700-705.

22. K.T. Holland, E. Ingham, W.J. Cunliffe, A Review The Microbiology of *Acne. J. Applied Bacteriology.* 51 (2) (1981) 195-215.

23. R. Summerly, H.J. Yardley, M. Raymond, A. Tabiowo, E. Ilderton, The lipid composition of sebaceous glands as a reflection of gland size. *British Journal of Dermatology.* 94 (1) (1976)45-53.

24. P.M. Quinton, Sweating and its Disorders. *Annual Review of Medicine.* 34 (1) (1983) 429-452.

25. D. Larry, G. Berglund, D. Richard, R. Gallagher, D. Preston, E. McNall, Simulation of the thermal effects of dissolved materials in human sweat. *Computers and Biomedical Research.* 6(2) (1973) 127-138.

26. G.W. Cage, S.M. Wolfe, R.H. Thompson, R.S.J. Gordon, Effects of water intake on composition of thermal sweat in normal human volunteers. *J. Applied Physiology.* 29 (1970) 687-690.

27. F. Bayford, Sweat. *Fingerprint Whorld.* 1 (1976) 42-43.

28. P. Hamilton. Amino-acids on Hands. *Nature.* 205 (1965) 284-285.

29. B. Hadron, F. Hanimann, P. Anders, H. Curtius, R. Halverson, Free Amino-acids in Human Sweat from Different Parts of the Body. *Nature.* 215 (99) (1967) 416-417.

30. J. Oro, H. Skewes, Free Amino-Acids on Human Fingers: The Question of Contamination in Microanalysis. *Nature.* 207 (1) (1965) 1042-1045.

31. K. Uyttendaele, M. De Groote, V. Blaton, H. Peeters, F. Alexander, Analysis of the proteins in sweat and urine by agarose-gel isotachophoresis. *J. Chromatography* 132 (2) (1977) 261-266.

32. T.C. Boysen, S. Yanagawa, F. Sato, K. Sato, A modified anaerobic method of sweat collection. *J. Applied Physiology.* 56 (5) (1984) 1302-1307.

33. K. Sato, The physiology, pharmacology, and biochemistry of the eccrine sweat gland. *Reviews of Physiology, Biochemistry and Pharmacology.* 79 (1977) 51-131.

34. W.C. Lobitz, H.L. Mason, Chemistry of palmar sweat: Vii. discussion of studies on chloride, urea, glucose, uric acid, ammonia nitrogen and creatinine. *Archives of Dermatology and Syphilology.* 57 (5) (1948) 907-915.

35. L. Förström, M.E. Goldyne, R.K. Winkelmann, Ige in Human Eccrine Sweat. *J. Inv. Derm.* 64 (3) (1975) 156-157.

36. M.J. Bailey, N.J. Bright, S. Hinder, B.N. Jones, R.P. Webb, R.S. Croxton, Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spec. *Anal Chem.* 84 (20) (2012) 8514-8523.

37. A. Girod, C. Weyermann, Lipid composition of fingermark residue and donor classification using GC/MS. Forensic Sci. Int. 238 (2014) 68-82.

38. B. Hadorn, F. Hanimann, P. Anders, H. Curtius, R. Halverson, Free Amino-acids in Human Sweat from Different Parts of the Body. *Nature.* 215 (99) (1965) 416-417.

39. A. Yamamoto, S. Serizawa, M. Ito, Y. Sato, Effect of Aging on Sebaceous Gland Activity and on the Fatty Acid Composition of Wax Esters. *J. Inv Derm*. 89 (5) (1987) 507-512.

40. P. Ramasastry, D.T. Downing, P.E. Pochi, J.S. Strauss, Chemical Composition of Human Skin Surface Lipids from Birth to Puberty. *J. Inv. Derm.* 54 (2) (1970) 139-144.

41. E. Jacobsen, J.K. Billings, R.A. Frantz, C.K. Kinney, M.E. Stewart, D.T. Downing, Age-Related Changes in Sebaceous Wax Ester Secretion Rates in Men and Women. *J. Inv. Derm.* 85 (5) (1985) 483-485.

42. W.J. Cunliffe, S. Shuster, Pathogenesis of Acne. *The Lancet.* 293 (7597) (1969) 685-687.

43. D.K. Williams, C.J. Brown, J. Bruker, Characterization of children's latent fingerprint residues by infrared microspectroscopy: Forensic implications. *Forensic sci. int.* 206 (1–3) (2011) 161-165.

44. R. Olsen, The chemical composition of palmar sweat. *Fingerprint and Identification magazine.* 53 (1972) 3-23.

45. A. Girod, C. Weyermann, Lipid composition of fingermark residue and donor classification using GC/MS. *Forensic Sci. Int*. 238 (2014) 68–82.

46. F. Rowell, K. Hudson, J. Seviour, Detection of drugs and their metabolites in dusted latent fingermarks by mass spectrometry. *Analyst*. 4 (2009) 701-707.

47. P.H.R. Ng, S. Walker, M. Tahtouh, B. Reedy, Detection of illicit substances in fingerprints by infrared spectral imaging. *Analytical & Bioanalytical Chemistry.* 394 (8) (2009) 2039-2048.

48. F. Rowell, J. Seviour, A.Y. Lim, C.G. Elumbaring-Salazar, J. Loke, J. Ma, Detection of nitro-organic and peroxide explosives in latent fingermarks by DART- and SALDI-TOF-mass spectrometry. *Forensic Sci. Int.* 221 (1–3) (2012) 84–91.

49. L. Sundar, F. Rowell, Detection of drugs in lifted cyanoacrylate-developed latent fingermarks using two laser desorption/ionisation mass spectrometric methods. *Analyst*. 139 (3) (2014) 633-642.

50. A.Y. Lim, J. Ma, Z. Ma, F. Rowell, Separation of fingerprint constituents using magnetic silica nanoparticles and direct on-particle SALDI-TOF-mass spectrometry. *Journal of Chromatography B.* 879 (23) (2011) 2244-2250.

51. R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese, Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids. *Rapid Communications in Mass Spectrometry*. 23 (19) (2009) 3031–3039.

52. L. Ferguson, R. Bradshaw, R. Wolstenholme, M. Clench, S. Francese, Two-Step Matrix Application for the Enhancement and Imaging of Latent Fingermarks. *Analytical Chemistry*. 83 (14) (2011) 5585–5591.

53. R. Bradshaw, R. Wolstenholme, R.D. Blackledge, M.R. Clench, L.S. Ferguson, S. Francese, A novel matrix-assisted laser desorption/ionisation mass spectrometry imaging based methodology for the identification of sexual assault suspects. *Rapid Communications in Mass Spectrometry*. 25 (3) (2011) 415–422.

54. S. Francese, R. Bradshaw, N. Denison, An update on MALDI mass spectrometry based technology for the analysis of fingermarks - stepping into operational deployment. *Analyst.* 142 (14) (2017) 2518–2546.

55. L.S. Ferguson, F. Wulfert, R. Wolstenholme, J.M. Fonville, M.R. Clench, V.A. Carolan, Direct detection of peptides and small proteins in fingermarks and determination of sex by MALDI mass spectrometry profiling. *Analyst.* 137 (20) (2012) 4686-4692.

56. A. Sullyman, Fingerprint test that reveals a criminal's lifestyle to be used in court 'in months'. *Independent.* (2017) online. Available at: http://www.independent.co.uk/life-style/gadgets-and-tech/news/fingerprint-test-criminal-lifestyle-profile-drugs-blood-courts-trials-maldi-mass-spectrometry-a7992621.html

57. K.A. Mountfort, H. Bronstein, N. Archer, S.M. Jickells, Identification of oxidation products of squalene in solution and in latent fingerprints by ESI-MS and LC/APCI-MS. *Analytical Chemistry*. 79 (7) (2007) 2650–2657.

58. R.M. Connatser, S.M. Prokes, O.J. Glembocki, R.L. Schuler, C.W. Gardner, S.A. Lewis, Toward Surface-Enhanced Raman Imaging of Latent Fingerprints. *J. Forensic Sci.* 55 (6) (2010) 1462–1470.

59. H.G.M. Edwards, D.M. Farwell, S. Rozenberg, Raman spectroscopic study of red pigment and fresco fragments from King Herod's Palace at Jericho. J*ournal of Raman Spectroscopy.* 30 (5) (1999) 361–366.

60. C.A.F. de Oliveira Penido, L. Silveira, M.T.T. Pacheco, Quantification of Binary Mixtures of Cocaine and Adulterants using Dispersive Raman and FTIR Spectroscopy and Principal Component Regression. *Instrumentation Science & Technology.* 40 (5) (2012) 441.

61. H.W. Han, X.L. Yan, R.X. Dong, G. Ban, K. Li, Analysis of serum from type II diabetes mellitus and diabetic complication using surface-enhanced Raman spectra (SERS). *Applied Physics B: Lasers and Optics.* 94 (4) (2009) 667–672.

62. J.S. Day, H.G. Edwards, S.A. Dobrowski, A.M. Voice, The detection of drugs of abuse in fingerprints using Raman spectroscopy II: cyanoacrylate-fumed fingerprints. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 60 (8–9) (2004) 1725–1730.

63. C. Cheng, T.E. Kirkbride, D.N. Batchelder, R.J. Lacey, T.G. Sheldon, In situ detection and identification of trace explosives by Raman microscopy. *J. Forensic sci.* 40 (1) (1995) 31–37.

64. D.K. Williams, R.L. Schwartz, E.G. Bartick, Analysis of Latent Fingerprint Deposits by Infrared Microspectroscopy. *Applied Spectroscopy.* 58 (3) (2004) 313–316.

65. N.J. Crane, E.G. Bartick, R.S. Perlman, S. Huffman, Infrared spectroscopic imaging for noninvasive detection of latent fingerprints. *J. Forensic Sci.* 52 (1) (2007) 48–53.

66. C. Ricci, N. Curum, K.L.A. Chan, S. Jickells, S. Kazarian, Chemical Imaging of Latent Fingerprint Residues. *Applied Spectroscopy*. 61 (5) (2007) 514–522.

67. A. Hemmila, J. McGill, D. Ritter, Fourier transform infrared reflectance spectra of latent fingerprints: a biometric gauge for the age of an individual. *J. Forensic sci.* 53 (2) (2008) 369–376.

68. A. Banas, K. Banas, M.B.H. Breese, J. Loke, S.K. Lim, B. Heng Teo, Detection of microscopic particles present as contaminants in latent fingerprints by means of synchrotron radiation-based Fourier transform infra-red micro-imaging. *Analyst.* 137 (15) (2012) 3459–3465.

69. A. Banas, K. Banas, M.B. Breese, J. Loke, S.K. Lim, Spectroscopic detection of exogenous materials in latent fingerprints treated with powders and lifted off with adhesive tapes. Anal. & Bioanal. Chemistry. 406 (17) (2014) 4173-4181.

70. Y. Mou, J.W. Rabalais, Detection and Identification of Explosive Particles in Fingerprints Using Attenuated Total Reflection-Fourier Transform Infrared Spectromicroscopy. *J. Forensic Sci.* 54 (4) (2009) 846-850.

CHAPTER 3. AIMS AND OBJECTIVES

The overall aim of this research was to investigate the dynamic nature of latent fingermark chemistry with a particular focus on temporal decomposition.

Despite recent advances in analytical instrumentation enabling a more in-depth understanding of fingermark chemistry there remains significant gaps in the literature. As such it was hypothesised that FTIR spectromicroscopy is ideally suited in providing a broad range of analytical information on latent fingermark chemistry and illicit substance detection within latent fingermarks, that could address some of these gaps.

Four areas of original research were identified which would contribute to a deeper understanding of fingermark chemistry. When combined these objectives test the hypothesis and deliver against the overall aim of the thesis, summarised in the diagram over the page.

The four objectives of this research were as follows:

Objective 1. To evaluate and critically review the advantages and limitations of Fourier Transform Infrared spectromicroscopy when compared to Gas Chromatography-Mass Spectrometry (GC-MS), the dominant analytical technique for the investigation of latent fingermark chemistry.

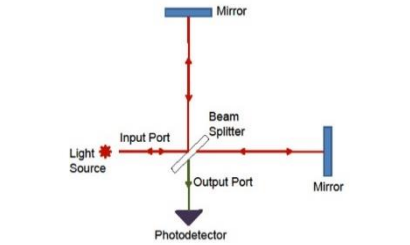
Objective 2. To determine whether moderate temperature variations affect latent fingermark chemistry in the immediate hours after deposition using FTIR spectromicroscopy.

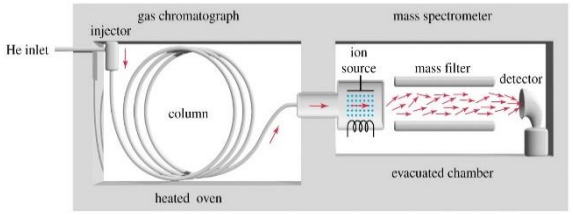
Objective 3. To investigate the possible intermolecular interactions that occur within latent fingermarks and their potential impact on degradation processes using FTIR spectroscopy.

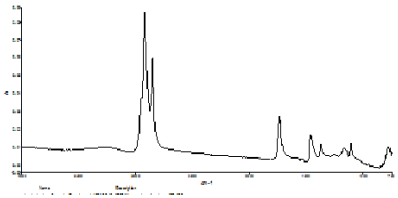
Objective 4. To detect and identify temporally degraded exogenous contaminants within latent fingermarks using FTIR spectroscopic imaging.

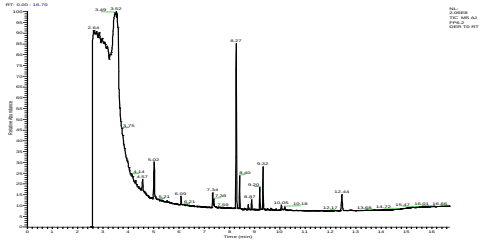
Diagrammatic summary of the objectives and workflow of research

FTIR **V's** GC-MS

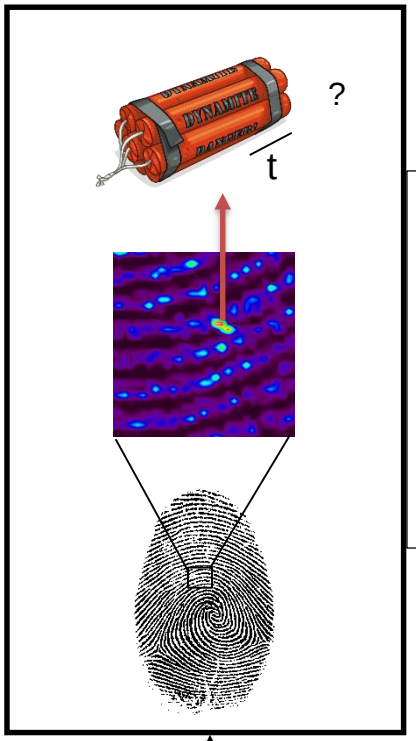




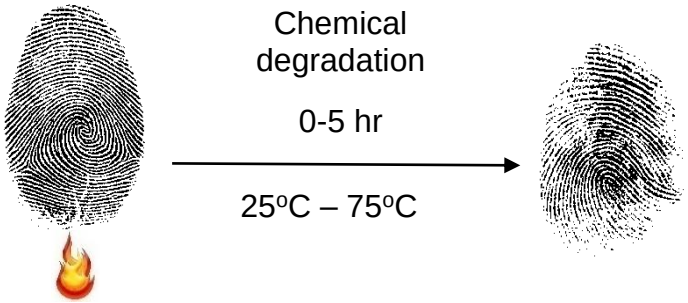




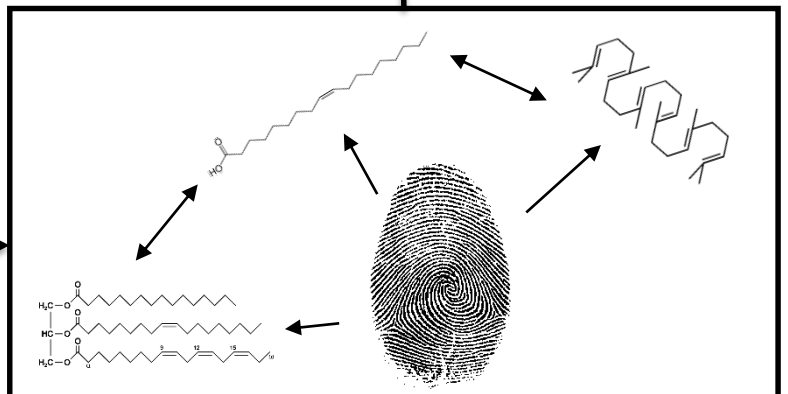
Objective 1: Evaluate the advantages and limitations of Fourier Transform Infrared (FTIR) spectromicroscopy when compared to Gas Chromatography-Mass Spectrometry (GC-MS).



Objective 4: To detect and identify exogenous contaminants within latent fingermarks over time using Fourier Transform spectroscopic imaging.



Objective 2: To determine whether moderate temperature variations affect latent fingermark chemistry in the immediate hours after deposition using FTIR spectromicroscopy.



Objective 3: To investigate the possible intermolecular interactions that occur within latent fingermarks and their potential impact on degradation processes using FTIR spectroscopy.

CHAPTER 4. PAPER ONE

(Submitted to *Journal of imaging* – under review*)*

The chemical analysis of latent fingermarks: Capabilities and limitations of FTIR spectroscopy, a GC-MS comparison

Authors

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4.1 Abstract

The capabilities of Fourier Transform Infrared (FTIR) spectroscopy as an analytical tool for the analysis of latent fingermarks were examined. Data were compared to the more established technique of gas chromatography-mass spectrometry (GC-MS) for the analysis of latent fingermark composition.

FTIR spectroscopy allowed for rapid, non-destructive analysis of latent fingermarks. Chemical imaging using FTIR spectromicroscopy visually demonstrated the intra-variability and distribution of components within latent fingermark deposits, although specific compound identification was limited.

Comparing ratios of certain functional groups to the antisymmetric and symmetric C-H stretch absorbance band (2924 cm^{-1} - 2840 cm^{-1}) showed a strong correlation between the relative quantities of some, but not all of those functional groups and the C-H stretch band.

GC-MS analysis identified the presence of specific amino acids, fatty acids, and squalene, and demonstrated quantitative intra-variability of these compounds from the fingermarks of the same individual. GC-MS was able to distinguish between *in vivo* lipid secretions and an exogenous synthetic lipid found in cosmetics. These results show that the orthogonal use of FTIR spectroscopy and GC-MS could provide a deeper understanding of fingermark chemistry.

Keywords

forensic science, latent fingermarks, chemical composition, FTIR microscopy, CGMS.

4.2 Introduction

Fingermark chemistry is complex, with significant variability both within and between individuals, is susceptible to significant changes over time, in different environmental conditions, and between children and adults [1-4]. Interest in fingermark chemistry has increased in the last decade primarily due to advances in analytical instrumentation, but despite this increase in research an in-depth understanding is yet to be achieved [4]. One analytical technique that has generated significant interest within in this field is Fourier Transform Infrared (FTIR) spectroscopy.

FTIR spectroscopic analysis is fast and requires no sample preparation, FTIR spectroscopic imaging is particularly attractive to forensic scientists and law enforcement agencies because of its ability to non-destructively analyse a sample for trace amounts of illicit substances. FTIR imaging, therefore, allows for better continuity of evidence through the forensic chain compared to the more conventional destructive analytical methods such as gas chromatography-mass spectrometry (GC-MS) [5-12] and matrix-assisted laser desorption ionisation (MALDI-MS) [5, 13-16]. FTIR imaging provides more compelling evidence to a lay jury than GC-MS or MALDI-MS, as it does not require interpretation of complex data or heavy reliance on expert opinion. The identification of any illicit substances in a subjects' fingermarks using FTIR imaging demonstrates an unequivocal visual link between the individual, the fingermark and the illicit substance that is far easier for a jury to understand.

Although FTIR spectroscopy has many attributes that are appealing to the forensic community, it is still broadly considered a research tool for the analysis of endogenous and exogenous compounds within fingermarks, as opposed to technique actively utilised by law enforcement agencies. This is in contrast to techniques such as GC-MS and MALDI-MS which are considered the gold standard for the chemical analysis of latent fingermarks. GC-MS has been used in the majority of investigations into latent fingermark chemistry, it not only provides detailed analysis of sebaceous and eccrine components present within fingermarks [1, 2, 4, 5, 12, 17] but is more practical, being readily accessible in most research facilities. Both FTIR and GC-MS analysis can offer unique insight into the composition of a fingermark and could arguably be considered as complementary techniques for fingermark analysis.

Although previous research has looked at the merits of various analytical techniques for the analysis of fingermarks [5], the aim of this study was to demonstrate the capabilities and limitations of FTIR spectromicroscopy and compare that to GC-MS, a more established analytical tool for such analysis.

4.3 Materials and Methods

4.3.1 Fingermark preparation - FTIR

All sample fingermarks were obtained from a single donor. Hands were first washed and dried thoroughly. Hands were then placed in latex gloves to protect them from contamination. After twenty minutes hands were removed from the gloves, the index finger was then drawn from the bridge of the nose, under the eye to the temple ten times to collect sebaceous secretions and to simulate natural grooming behaviour, and placed directly onto a CaF_2 Infrared (IR) microscope slide (10 mm x 10 mm, Crystran Ltd). In total twenty sample fingermarks were prepared as above. All fingermarks were laid down between 9am and 11am to avoid significant diurnal variations in composition, allowing twenty minutes between depositions for sebaceous and eccrine recovery. For each fingermark the same index finger was used. All fingermarks were stored at room temperature and humidity for the duration of the experiment. All fingermarks were destroyed at the end of each day and the process repeated for each additional day of testing, this ensured fingermarks were fresh and analysis was not carried out on inadvertently aged fingermarks.

4.3.2 FTIR Microscopy

The latent fingermarks prepared as above were analysed using a Perkin® Elmer Spectrum™ Spotlight 200 FTIR imaging System equipped with a liquid-nitrogen cooled MCT linear array detector. Data was analysed using Perkin Elmer software, Spectrum® (v10.02.00), variations in spectra were processed using peak height calculations. For each fingermark six deposits were identified and analysed at random to ensure all components of the latent fingermarks were sampled.

Spectra of fingermark deposits were collected in transmission mode within 4000 to 750 cm^{-1} spectral range with 10 scans per pixel at 4 cm^{-1} spectral resolution and 10 µm spatial resolution, using a 100 x 100 µm aperture.

4.3.3 Fingermark preparation – GC-MS

Fingermarks were generated in an identical manner to FTIR preparation and deposited on acetone washed coverslips (22 mm x 22 mm Chance propper Ltd) cut in half to fit a screw top vial (2 ml, Merck UK). There was a minimum of twenty minutes between fingermark depositions to allow recovery of glandular secretions, in total twenty samples were prepared as above. All fingermarks were laid down using the same index finger.

Once fingermark samples were deposited on the coverslips they were placed into the screw top vial, each vial was marked with the fingermark number and extraction technique.

To extract the fingermark 1-chlorononane (30 µL of a 0.078 mg/mL solution in hexane) was added to 200 µl of the extraction agent dichloromethane (>99.9% pure), and 50 µl N-methyl-N-(trimethylsilyl)-triflouroacetamide (MSTFA) derivatisation agent. MSTFA alters the properties of compounds, increases their volatility making them more suitable for GC analysis. The vials were sealed with the screw cap, shaken and left to stand at room temperate for 15 minutes, shaken intermittently.

After 15 minutes the solution was reduced under a stream of nitrogen until the solution evaporated and only the solute remained. 100 µl of dichloromethane was added and 75 µl of solution was transferred to 300 µl glass autosampler vials (Thermo Fisher Scientific, UK) and shaken several times to mix. The samples were then analysed by GC-MS.

Blank coverslips were taken through the same preparation procedure as above to act as controls.

4.3.4 GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on a ThermoQuest GC AS 800 autosampler, Fisons GC model GC8000^{top} with AS 800 liquids auto sampler directly coupled with a Fisons Voyager MS (quadrupole, EI+) operated in full scan mode. 1 µl of sample was injected in via an AS800 autosampler. The column was a factor four VF-5MS (15 m length, 0.25 mm id x 0.25 µm film

thickness) from Varian. The injector was set at 250^{o}C and the MS transfer line temperature at 250^{o}C.

Helium was used as the carrier gas at a flow rate of 2.40 ml/min. The MS detector operated at 170V, emission 350 µA scanning between 15 and 526 amu at 2.1 scans/ s^{-1}. The column was held at 80^{o}C for 3 min after injection and the programmed to rise at 20^{o}C/min to 300^{o}C, and held at this temperature for 15 min. All mass spectra were obtained using electron ionisation (70 eV). Analysis software was Xcalibur from Thermo Scientific with National Institute of Standards and Technology (NIST) spectral library.

4.3.5 Reagents

Control chemicals: 1-chlorononane (98%, Fluka); cholesterol (99%, Fluka), MSTFA, were all purchased from Sigma-Aldrich Chemical Company and used without further purification. Solvents: acetone and dichloromethane were purchased from Fisher Chemicals.

4.3.5.1 Identification and quantification

Bands detected from the mass spectrum were identified through library matching against the NIST library of Mass spectra. Relative quantification was achieved by peak area ratio comparison to chlorononane.

4.4 Results and Discussion

4.4.1 FTIR - Chemical Mapping

FTIR spectra identify the functional groups of compounds present within a sample, with FTIR spectromicroscopy its possible to visually display the distribution of specific functional groups using chemical mapping. It is possible, for example, to display the alkenes (3100-3000 cm^{-1}) of unsaturated lipids and the carboxylic acid groups (1760-1690 cm^{-1}) in sebum deposits within latent fingermarks. These images demonstrate the relative abundance and distribution of particular compounds within a fingermark and can be used to look at the relationship between fingermark components by obtaining specific IR spectra from the chemimaps.

Figure 16 shows the difference in average absorbance, and the distribution of alkenes, and carboxylic acid groups from two different fingermarks from the same individual deposited on two consecutive days (figure 16A & B). These fingermarks were taken within the same thirty-minute time period on each day and were natural deposits, i.e. no simulated grooming behaviour. As there are no sebaceous glands on the palms of the hands (or soles of the feet), sebaceous secretions found in fingermarks are deposited onto the fingertips by natural grooming behaviour, typically face touching [3, 4, 8], and therefore can vary both diurnally and between individuals. Figure 16B shows a greater average absorbance and therefore higher relative proportion of fingermark components than 16A, so, as would be expected, there is a higher concentration of unsaturated lipids (alkenes), and carboxylic acid groups, which are found in various fingermark components such as fatty acids and amino acids.

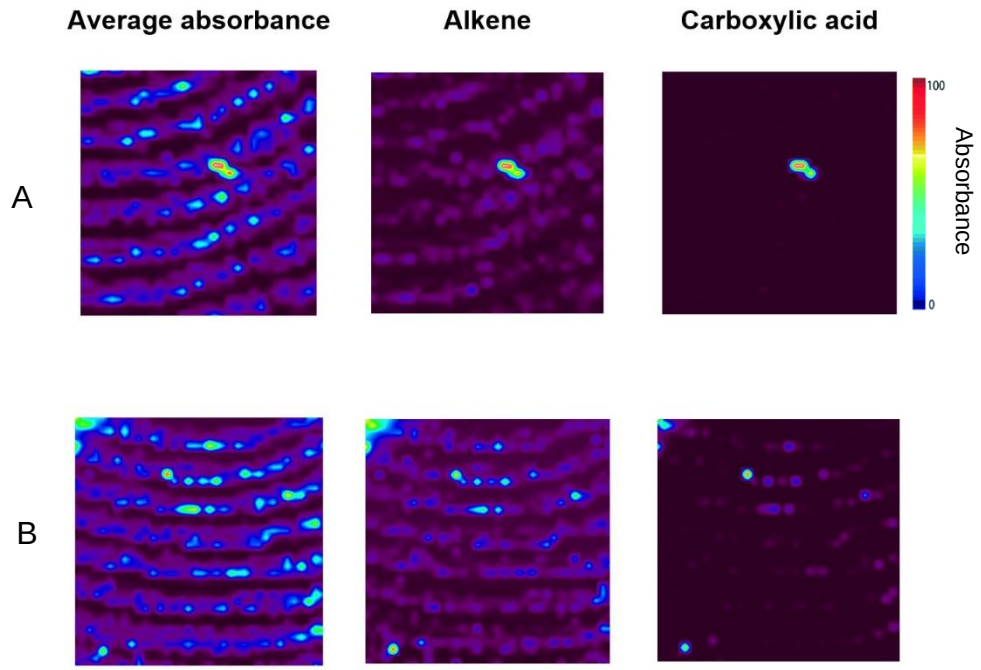

Figure 16: Chemimap images of the variations in sebum deposits between two latent fingermarks from the same individual.

4.4.2 FTIR - Relative quantification

The majority of components that make up a latent fingermark have been well documented using various analytical techniques, not least variations of mass spectrometry [1-14, 15-22]. As mentioned previously, spectroscopic techniques too have reported the chemical makeup of latent fingermarks, both Raman and FTIR spectroscopy, in particular, have been utilised to study the exogenous and endogenous components present within latent fingermarks [23-34]. Figure 17 shows a typical FTIR spectrum of a fresh natural fingermark.

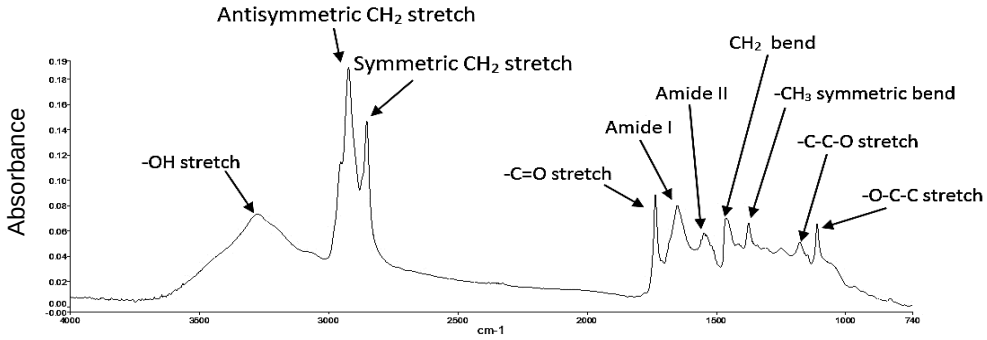

Figure 17: A typical FTIR spectrum of a natural latent fingermark.

The spectrum contains the principal components that make up a latent fingermark, i.e. sebum and eccrine sweat [3, 4], with the key functional groups labelled as consistent with the literature. Figure 17 shows absorbance bands at 3300 cm^{-1} (O-H stretch, water, protein), 2924 cm^{-1} and 2840 cm^{-1} (antisymmetric and symmetric C-H stretch of CH_2 groups), 1736 cm^{-1} (C=O stretch, saturated ester), 1654 cm^{-1} (I amide C=O stretch), 1540 cm^{-1} (II amide N-H in plane bend, C-N stretch), 1464 cm^{-1} (CH_3 asymmetric bend), 1374 cm^{-1} (CH_3 symmetric bend), 1164 cm^{-1} (C-C-O stretch, saturated ester), 1110 cm^{-1} (O-C-C stretch, saturated ester).

The dominant band in an FTIR spectrum of a latent fingermark is the antisymmetric and symmetric C-H stretch of CH_2 groups at 2924 cm^{-1} - 2840 cm^{-1}. This band is the sum of the organic components that make up a latent fingermark, Amino acids, fatty acids, triglycerides, wax esters, sterols, sterol esters, squalene, peptides, are all organic molecules, and each molecule ranging from two to over thirty carbon atoms [2-4, 7, 28, 33]. The only exception to this is the presence of water and minimal amounts of inorganic salts secreted from eccrine glands. It is therefore reasonable to expect that there is a strong correlation between the concentration of the symmetric and antisymmetric C-H stretch band of CH_2 groups and all the other bands in the spectrum that identify the various functional groups from organic molecules within a fingermark. These groups would include the saturated ester carbonyl group (C=O) at 1740 cm^{-1}, the amide I & II groups of peptide bonds (1541 cm^{-1}), the methyl group (R-CH_3) of aliphatic carbon chains at 1464 cm^{-1}, and the C-C-O saturated ester group at 1164 cm^{-1}. The antisymmetric and symmetric C-H stretch of CH_2 groups could, therefore, be considered to be the sum of these (and other) organic components.

The figures below show the correlation between the antisymmetric and symmetric C-H stretch of CH_2 groups and three of these other functional group bands.

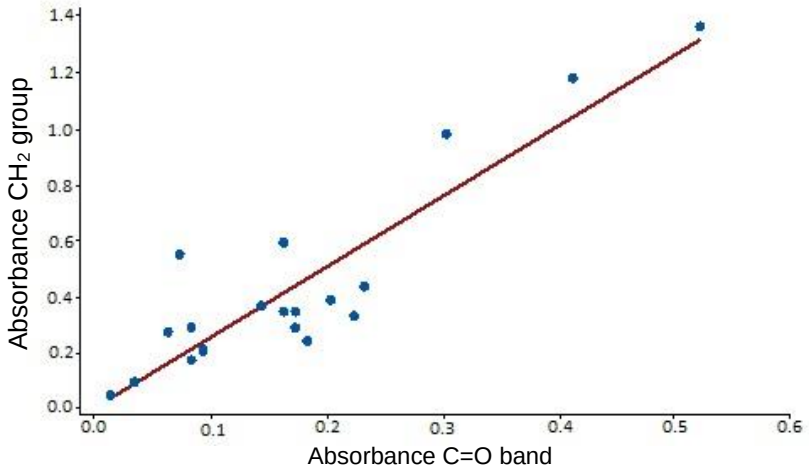

Figure 18: Relationship between the antisymmetric & symmetric C-H stretch of CH_2 groups (2924 – 2840 cm^{-1}) and the carbonyl stretch band (1740 cm^{-1}) of a latent fingermark (n=20)

Figure 18 shows a strong correlation between the concentration of antisymmetric and symmetric C-H stretch of CH_2 groups and the carbonyl functional group band with a correlation of R=0.92 (*p*-value 0.00).

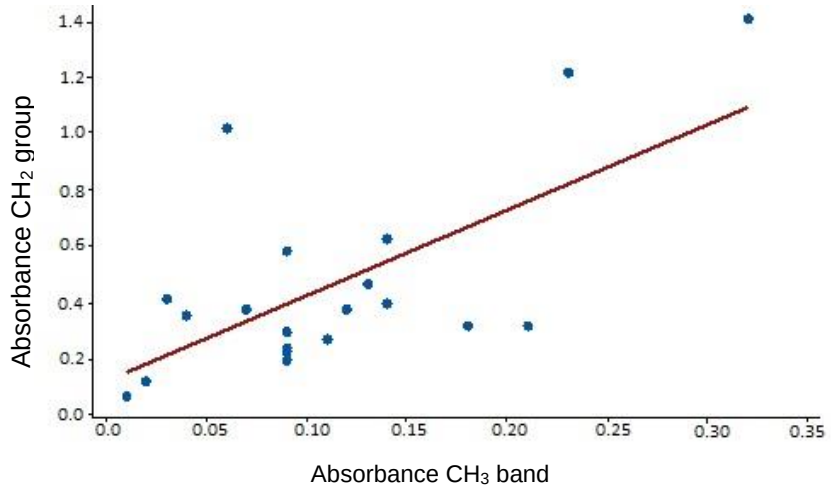

Figure 19: Relationship between the antisymmetric & symmetric C-H stretch of CH_2 groups (2924 – 2840 cm^{-1}) and the CH_3 bend (1464 cm^{-1}) (n=20)

Figure 19 shows a general trend of an increase in quantity of methyl groups as the concentration of the 2924 – 2840 cm^{-1} CH_2 band increases, but there is a significant degree of scatter and less correlation (R=0.66, *p*-value = 0.002) than that of the carbonyl band.

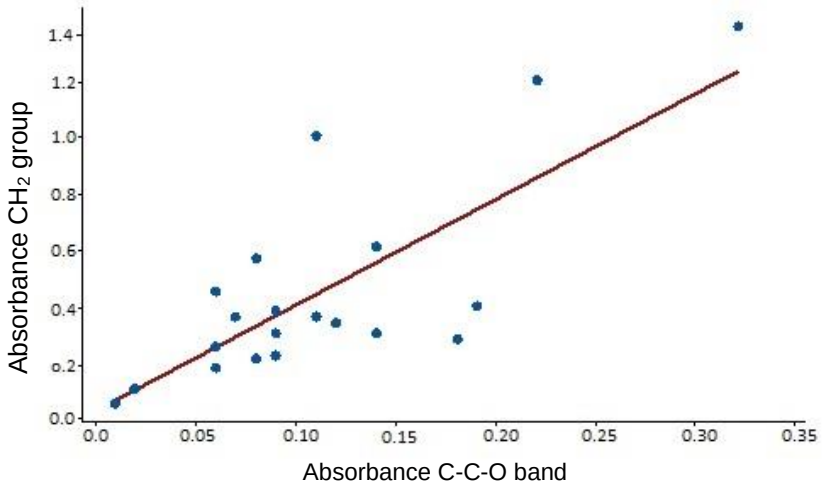

Figure 20: Relationship between the antisymmetric & symmetric C-H stretch of CH_2 groups (2924 cm^{-1} - 2840 cm^{-1}) and the C-C-O stretch band (1246 cm^{-1}) (n=20)

Figure 20 again shows a correlation between the antisymmetric & symmetric C-H stretch band and the C-C-O functional group but with significant scatter, (R = 0.77, *p*-value <0.01).

Figures 18-20 show there is a correlation between the concentration of the antisymmetric & symmetric C-H stretch of CH_2 groups at 2924 cm^{-1} - 2840 cm^{-1} and the associated functional groups, however, in some cases these correlations are not as strong as might be expected. This suggests that an increase in the total organic component within a latent fingermark does not necessarily correspond to an increase in every component part of that fingermark (i.e. fatty acids, triglycerides, wax esters and so on). For example, as shown in figure 19, an increase in concentration of the overall organic composition of a latent fingermark (2924 cm^{-1} - 2840 cm^{-1}) does not consistently translate into an increase in short aliphatic carbon chains (1464 cm^{-1}). In contrast, there is a strong correlation between the overall organic composition within a latent fingermark and the concentration of saturated esters (figure 18).

4.4.3 GC-MS

Figure 21 shows the results of GC-MS analysis of the donor's latent fingermarks. It is a typical chromatogram of a fresh fingermark, note the presence of long chain fatty acids known to be present in latent fingermarks [2, 7, 11, 17, 19, 35]. The most commonly occurring being palmitic acid (base peak), dodecanoic acid, oleic acid and stearic acid. Short chain fatty acids such as octanoic acid occurred less frequently, as previously reported [7, 17, 20]. Squalene was observed in all samples analysed. Cholesterol was only reliably observed in four of the twenty samples and at very low concentration. The amino acid glycine was consistently observed in fourteen of the twenty fresh fingermarks analysed.

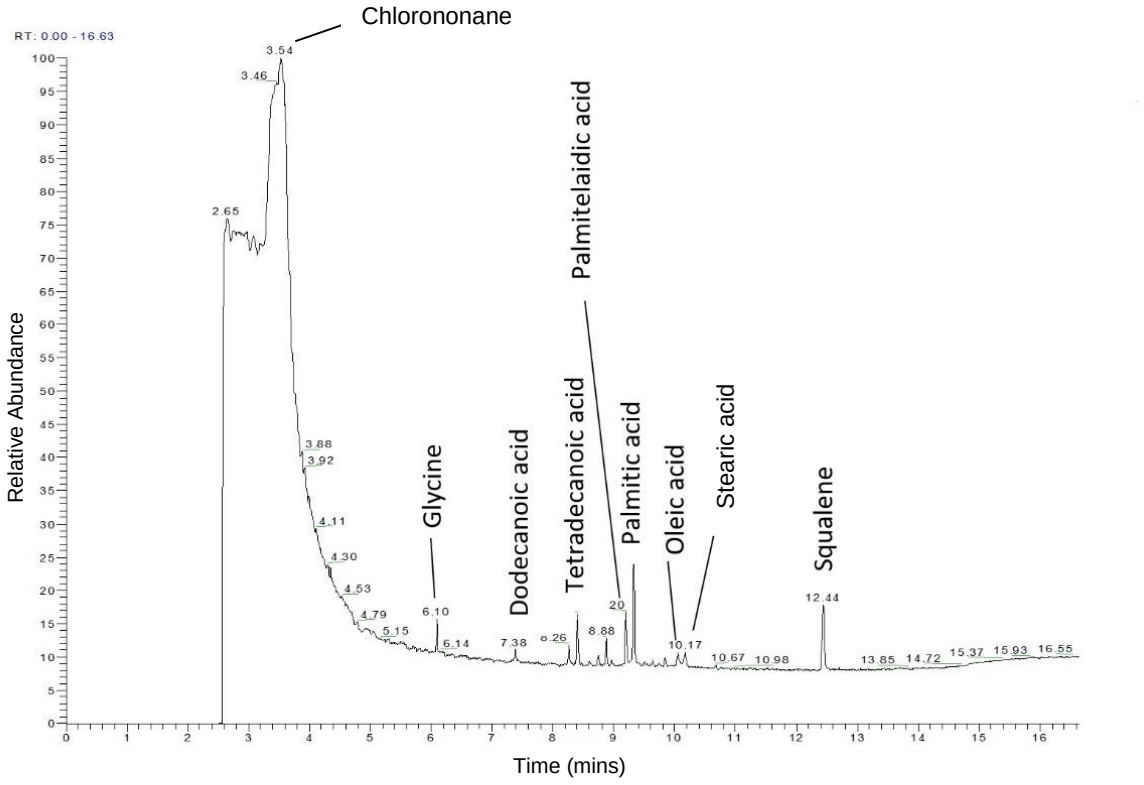

Figure 21: A typical chromatogram of a latent fingermark from this study

4.4.4 Relative Quantification

Results were quantified by calculating mean peak area ratio relative to an internal standard, chlorononane (CN). Table 5 shows the seven most consistently identified components of the donor's fingermarks as a percentage of CN. As was consistent with the results shown by FTIR spectroscopic imaging, the quantity of lipid deposits varied considerably between samples.

% Peak area ratios /chlorononane (CN)							
Compound	*Glycine/CN*	*Tetradecanoic acid/CN*	*Palmitelaidic acid/CN*	*Palmitic acid/CN*	*Oleic acid/CN*	*Steric acid/CN*	*squalene/CN*
Mean %	**0.023**	**0.073**	**0.067**	**1.38**	**0.21**	**0.16**	**1.07**
standard Dev.	**0.12**	**0.44**	**0.47**	**0.86**	**0.17**	**0.11**	**0.37**

Table 5: Average percentage quantities of the seven most consistently detected lipids as compared to chlorononane (CN)

Figure 22 shows a boxplot diagram of the relative quantities of the most abundant compounds (tetradecanoic acid, palmitelaidic acid, palmitic acid, oleic acid, stearic acid, glycine and squalene) relative to CN from all twenty samples. The graph shows that palmitic acid is consistently present in the highest concentrations within the sampled fingermarks, and with the broadest range from 0% to 2.33%. Squalene was the next most abundant compound, with less variation but a broad upper range. The consistent presence of squalene within each sample was observed in previous studies [1, 2, 7, 10, 11, 21] and is a major reason why it is a crucial molecule for biometric analysis, and estimating the time since deposition of a fingermark.

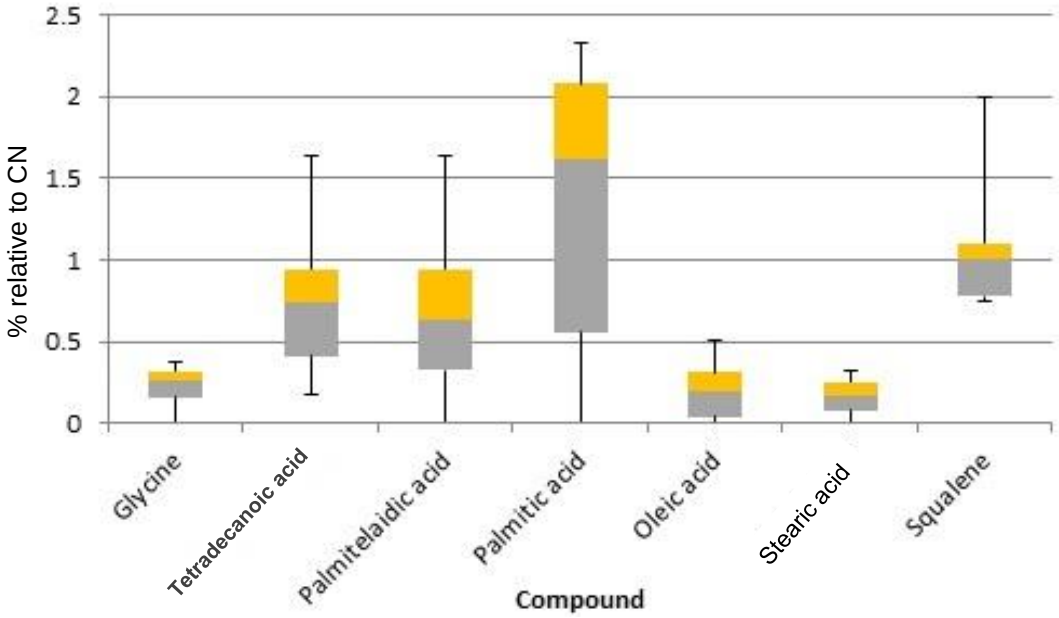

Figure 22: A boxplot diagram of the seven most abundant compounds found in the twenty latent fingermarks from this study

Figure 23 shows a chromatogram similar to that of figure 21 but with the base peak being isopropyl myristate (8.27 min), not palmitic acid (9.32 min). Isopropyl myristate ($C_{17}H_{34}O_2$) is an oil not naturally produced by the body but a synthetic compound similar to the naturally occurring fatty acid myristic acid ($C_{14}H_{28}O_2$) and is used in a number of cosmetics and moisturisers. This particular ester was present within a moisturiser (Doublebase, Dermal Laboratories, UK) used by the donor.

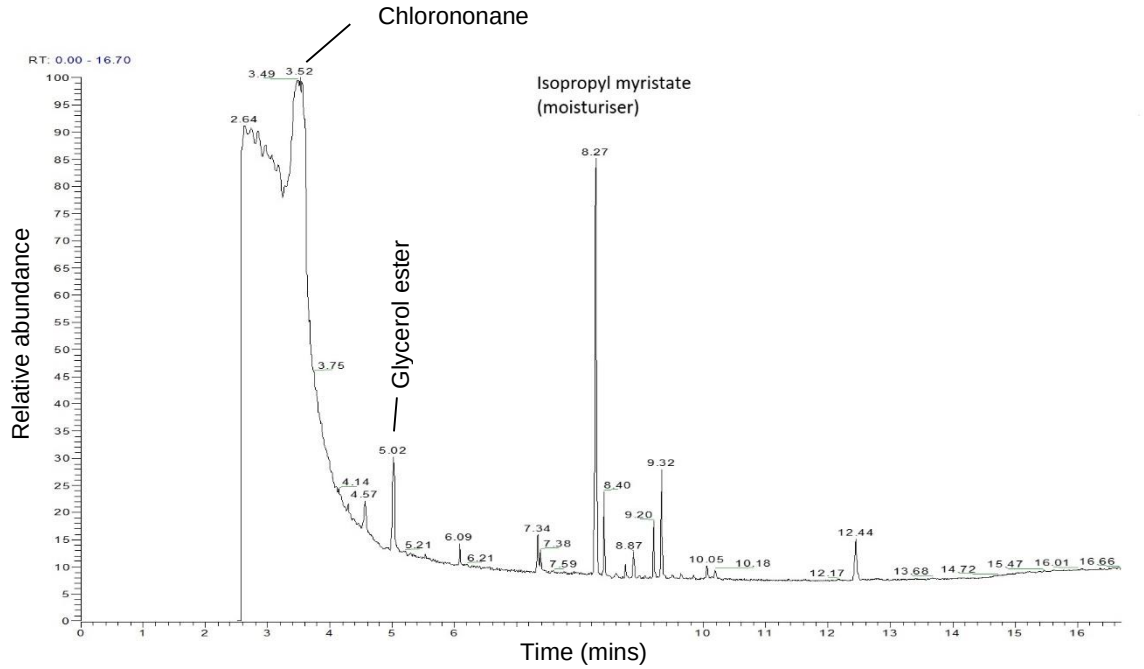

Figure 23: A chromatogram of a fingermark containing the synthetic oil isopropyl myristate.

This study aimed to look at the capabilities of FTIR spectromicroscopy as an imaging and analytical tool for the analysis of latent fingermarks. FTIR spectroscopic data was also compared to samples analysed by GC-MS, considered the gold standard for fingermark analysis. The composition of latent fingermarks has been comprehensively studied using GC-MS, and to a lesser extent using FTIR, but a direct comparison of the two techniques has never been investigated.

The results obtained from both the GC-MS and FTIR analysis have demonstrated the complimentary nature of these two techniques. Using FTIR spectroscopic imaging to chemically map latent fingermarks visually demonstrated the intra-variability in composition between fingermark samples (figure 16), and is consistent with the GC-MS data in this study and from previous studies (figure 22) [2, 7, 11]. This variation between samples from a single donor demonstrates the challenges of using latent fingermarks as biomarkers for age and gender determination, and supports research carried out by Asano et al. [6]. This study reported that analysis of fingermark composition for gender determination was statistically insignificant due to the variability between fingermarks, although more recent studies using MALDI-TOF-MS have had more success [37].

A further complicating factor in using latent fingermarks as biomarkers, as reported by Mountfort et al. [21] and Archer et al. [7], is that squalene, and other lipids, [11] are prone to shortening and oxidation over time. This oxidation results in the formation of degradation products such as fatty acid esters, peroxides and hydroperoxides, particularly in light conditions [7]. It is likely that over time the quantities of short-chain fatty acids, such as pentandioic and hexandioic acid [11], within a fingermark (as well as a number of intermediary oxidation products) will increase as components such as triglycerides, unsaturated lipids and squalene continue to degrade. These dynamic changes that occur within latent fingermarks post-deposition could confuse any attempts to obtain accurate information about an individual from their endogenous fingermark chemistry. However, the ratio of certain components to their degradation products, such as the ratio of squalene (SQ) to SQ hydroperoxides, could perhaps be used to more accurately estimate the time since deposition of a fingermark. FTIR spectroscopy alone would, however, be unable to accurately detect these changes as many of the

degradation products are structurally similar to pre-existing components and would therefore be lost within the same spectral regions.

This study demonstrated that there is a correlation between the concentration of the antisymmetric & symmetric C-H stretch band of CH_2 groups (2924 - 2840 cm^{-1}), the combination of all organic materials within a latent fingermark, and other functional groups, namely -C=O stretch (1736 cm^{-1}), -CH_3 asymmetric bend (1464 cm^{-1}), C-C-O stretch (1180 cm^{-1}). This correlation, however, was not as consistently significant as would be expected (Figures 18-20). There was significant variation in the concentrations of these functional groups when compared to the concentration of the antisymmetric & symmetric C-H stretch band of CH_2 groups. This suggests that an increase in the total organic content of a latent fingermark does not necessarily correspond to an increase in every component part of that fingermark. This also suggests that the CH_2 band at 2924 cm^{-1} – 2840 cm^{-1} is comprised of more than just the organic molecules containing these identifiable functional groups. This band therefore must contain other organic molecules with no or very few functional groups, molecules which are effectively undifferentiable to FTIR. For example, the presence of the metabolic steroid precursor squalene, a large organic molecule (C30), consists entirely of C-H bonds and makes up approximately 10% of sebaceous secretions [10]. Therefore, squalene will contribute significantly to the antisymmetric and symmetric C-H stretch band at 2924-2840 cm^{-1}. Due to the lack of any other functional groups squalene will not affect the absorbance bands generated by functional groups present in fatty acids, wax esters, sterols or glycerides. Cholesterol (C27), making up about 2% of sebaceous secretions, will also contribute to the antisymmetric and symmetric C-H bands in an FTIR spectrum, with only a minimal influence on the OH stretch band at 3400 cm^{-1}.

The FTIR data obtained in this study demonstrated that there is a stronger correlation between the CH_2 band at 2924 - 2840 cm^{-1} to the carbonyl (1740 cm^{-1}) and C-C-O bands (1246 cm^{-1}), than that of the methyl groups (1464 cm^{-1}). This stronger association is likely due to the sample fingermarks in this study being donated by an adult. Esters contain carbonyl groups, and it is known that fatty acids and wax esters increase during and after puberty, and are found in higher concentrations in adult fingermarks [1, 9]. The weaker

correlation between the C-H stretch band at 2924-2840 cm^{-1} and the methyl asymmetric bend is harder to explain and will need further investigation, as there is a higher proportion of CH_3 groups in adult sebum due to the more highly branched lipids, wax esters and branched fatty acids [1].

Further investigation into the correlation between the CH_2 band at 2924-2840 cm^{-1} and other functional groups, such as the carbonyl or methyl bands, in adult and children's latent fingermarks could, when combined with other markers, produce an effective presumptive identifier to differentiate these fingermarks at a crime scene. The ability to quantify the concentrations of components such as fatty acids or wax esters within a fingermark using GC-MS may allow for a better understanding of the contribution of these compounds to the less defined antisymmetric and symmetric C-H stretch band in an FTIR spectrum. With further research, this could be used to generate a more detailed model of the overall molecular composition of these currently loosely defined bands. This, in turn, would make any significant variations in the composition of these bands easier to identify.

In situ fingermarks found at a crime scene would vary considerably depending on the grooming behaviour of the individual. The amount of contact with sebaceous secretions on the face or neck would dramatically increase the concentrations of fatty acids, wax esters, glycerides, sterols and sterol esters within the fingermark and would rarely be consistent between different fingermarks even from the same individual, as this study has shown. The addition of synthetic oils and lipids found in many cosmetics would compound this variation between fingermarks even further. The use of a spectroscopic technique such as FTIR cannot reliably distinguish between naturally occurring sebaceous secretions and synthetic organic compounds found in cosmetics, but the presence of these synthetic compounds could dramatically alter an FTIR spectrum [36]. The identification of isopropyl myristate (a synthetic oil commonly used in moisturisers) by GC-MS analysis in this study highlights this issue and demonstrates the advantages of an orthogonal analytical technique in certain circumstances when analysing latent fingermarks using FTIR spectroscopy.

4.5 Conclusion

This study has demonstrated both the benefits of using FTIR spectromicroscopy as an orthogonal technology and its limitations as a stand-alone analytical tool. FTIR spectroscopic imaging is capable of chemically and spatially identifying the functional groups that make up a latent fingermark without destroying the sample. Chemical mapping also visually demonstrated the variability in component concentration within latent fingermark deposits, even from the same individual, and supported the GC-MS data which showed broad quantifiable variation in composition within the sample fingermarks analysed. FTIR analysis demonstrated that concentration ratios of certain functional groups compared to the antisymmetric & symmetric C-H stretch band of CH_2 groups showed a correlation, but not always as strong as might be expected. With further research, these ratios may enable the differentiation of children's and adult's fingermarks.

With a better understanding of chemical variations between latent fingermarks, FTIR chemical imaging could potentially be used as a presumptive test to estimate the age of a fingermark, as it is known that the composition of latent fingermarks varies depending on time since deposition. FTIR spectroscopy can, however, only qualify the general composition of a latent fingermark, and the variability in composition between fingermarks. FTIR analysis cannot provide information on specific compounds such as a specific fatty acid, and, unlike GC-MS, cannot reliably distinguish between naturally occurring components that make up a latent fingermark and synthetic oils or lipids from cosmetics that are present within that fingermark from grooming behaviour.

4.6 References

1. K.M. Antoine, S. Mortazavi, L.M. Miller, Chemical differences are observed in children's versus adults' latent fingerprints as a function of time. *J. Forensic Sci*. 55 (2010) 513-518.

2. R.S. Croxton, M.G. Baron, D. Butler, T. Kent, V.G. Sears, Variation in amino acid and lipid composition of latent fingerprints. *Forensic Sci. Int*. 199 (2010) 93-102.

3. S. Cadd, M. Islam, P. Manson, S. Bleay, Fingerprint composition and aging: A literature review. *Sci. Justice*. 55 (2015) 219-238.

4. A. Girod, R. Ramotowski, C. Weyermann, Composition of fingermark residue: A qualitative and quantitative review. *Forensic Sci. Int*. 223 (2012) 10-24.

5. M.J. Bailey, N.J. Bright, S. Hinder, Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spec. *Anal. Chem*. 84 (2012) 8514-8523.

6. K.G. Asano, C.K. Bayne, K.M. Horsman, M.V. Buchanan, Chemical composition of fingerprints for gender determination. *J. forensic sci.* 47 (4) (2002) 805–807.

7. N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells, Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface. *Forensic Sci. Int*. 154 (2005) 224-239.

8. C. Weyermann, C. Roux, C. Champod, Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis. *J. Forensic Sci*. 56 (2011) 102-108.

9. M.V. Buchanan, K.G. Asano, A. Bohanon, Chemical Characterization of fingerprints from adults and children. *Int. Soc. Opt. Eng*. 2941 (1996) 89-95.

10. R. Ramotowski, *Composition of Latent Print Residues, in: Advanced in Fingerprint Technology, 2nd Ed.* (Lee. H, Gaensslen. R, eds.). London: CRC Press (2001

11. G.M. Mong, C.E. Petersen, T.R.W. Clauss, Advanced Fingerprint Analysis Project - Fingerprint Constituents. Vol PNNL-13019. Pacific Northwest National Laboratory: U.S. Department of Energy. (1999).

12. A. Koenig, A. Girod, C. Weyermann, Identification of wax esters in fingermark residues by GC/MS and their potential use as aging parameters. *J. Forensic Identification*. 61(6) (2011) 652-76.

13. S. Francese, R. Bradshaw, L.S. Ferguson, R. Wolstenholme, M.R. Clench, S. Bleay, Beyond the ridge pattern: Multi-informative analysis of latent fingermarks by MALDI mass spectrometry. *Analyst*. 138 (15) (2013) 4215-4228.

14. L. Ferguson, R. Bradshaw, R. Wolstenholme, M. Clench, S. Francese, Two-Step Matrix Application for the Enhancement and Imaging of Latent Fingermarks. *Analytical Chemistry*. 83 (14) (2011) 5585–5591.

15. R. Wolstenholme, S. Francese, R. Bradshaw, Study of lipid distribution and degradation in latent fingerprints by spectroscopic imaging techniques. *Science & Justice*. 50 (1) (2010) 37-38.

16. R. Bradshaw, W. Rao, R. Wolstenholme, M.R. Clench, S.Bleay, S. Francese, Separation of overlapping fingermarks by matrix assisted laser desorption ionisation mass spectrometry imaging. *Forensic Sci Int*. 222 (1–3) (2012) 318-326.

17. A. Girod, C. Weyermann, Lipid composition of fingermark residue and donor classification using GC/MS. *Forensic Sci Int*. 238 (2014) 68-82.

18. R. Bradshaw, S. Bleay, R. Wolstenholme, M.R. Clench, S. Francese, Towards the integration of matrix assisted laser desorption ionisation mass spectrometry imaging into the current fingermark examination workflow. *Forensic Sci Int*. 232 (2013) 111-124.

19. B. Emerson, B. Durham, J. Gidden, J.O. Lay, Laser desorption/ionization time-of-flight mass spectrometry of triacylglycerols and other components in fingermark samples. *J Forensic Sci*. 56 (2) (2011) 381-389.

20. A.Y. Lim, J. Ma, Z. Ma, F. Rowell, Separation of fingerprint constituents using magnetic silica nanoparticles and direct on-particle SALDI-TOF-mass spectrometry. *Journal of Chromatography B.* 879 (23) (2011) 2244–2250.

21. K.A. Mountfort, H. Bronstein, N. Archer, S.M. Jickells, Identification of oxidation products of squalene in solution and in latent fingerprints by ESI-MS and LC/APCI-MS. *Anal. Chem*. 79 (2007) 2650-2657.

22. D. Noble, Vanished into thin air. The search for children's fingerprints. *Anal. Chem*. 67 (1995) 435A-438A.

23. A. Banas, K. Banas, M.B. Breese, J. Loke, S.K. Lim, Spectroscopic detection of exogenous materials in latent fingerprints treated with powders and lifted off with adhesive tapes. *Analytical & Bioanalytical Chemistry*. 406 (17) (2014) 4173-81.

24. C. Ricci, N. Curum, K.L. Andrew Chan, S. Jickells, S. Kazarian, P. Phiriyavityopas, Chemical Imaging of Latent Fingerprint Residues. *Appl Spectrosc*. 61 (2007) 514-522.

25. T. Chen, Z.D. Schultz, I.W. Levin, Infrared spectroscopic imaging of latent fingerprints and associated forensic evidence. *Analyst*. 134 (9) (2009) 1902-1904.

26. R.M. Connatser, S.M. Prokes, O.J. Glembocki, R.L. Schuler, C.W. Gardner, S.A. Lewis, Toward Surface-Enhanced Raman Imaging of Latent Fingerprints Toward Surface-Enhanced Raman Imaging of Latent Fingerprints. *J. Forensic Sci.* 55 (6) (2010) 1462–1470.

27. N.J. Crane, E.G. Bartick, R.S. Perlman, S. Huffman, Infrared spectroscopic imaging for noninvasive detection of latent fingerprints. *J. Forensic Sci*. 52 (2007) 48-53.

28. P. Fritz, W. van Bronswjik, K. Lepkova, Infrared microscopy studies of the chemical composition of latent fingermark residues. *Microchem. J*. 111 (2013) 40-46.

29. A. Hemmila, J. McGill, D. Ritter, Fourier transform infrared reflectance spectra of latent fingerprints: a biometric gauge for the age of an individual. *J. Forensic sci.* 53 (2) (2008) 369–376.

30. P.H.R. Ng, S. Walker, M. Tahtouh, B. Reedy, Detection of illicit substances in fingerprints by infrared spectral imaging. *Anal. Bioanal. Chem*. 394 (2009) 2039.

31. M. Tahtouh, J.R. Kalman, C. Roux, B.J. Reedy, C. Lennard, The detection and enhancement of latent fingermarks using infrared chemical imaging. *J Forensic Sci*. 50 (1) (2005) 64-72.

32. M. Tahtouh, P. Despland, R. Shimmon, J.R. Kalman, B.J. Reedy, The Application of Infrared Chemical Imaging to the Detection and Enhancement of Latent Fingerprints: Method Optimization and Further Findings. *J. Forensic Sci*. 52 (2007) 1089-1096.

33. D.K. Williams, R.L. Schwartz, E.G Bartick, Analysis of Latent Fingerprint Deposits by Infrared Microspectroscopy. *Appl. Spectrosc*. 58 (2004) 313-316.

34. D.K. Williams, C.J. Brown, J. Bruker, Characterization of children's latent fingerprint residues by infrared microspectroscopy: Forensic implications. *Forensic Sci. Int*. 206 (2011) 161-165.

35. R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese, Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids. *Rapid Commun. Mass Spectrom*. 23 (2009) 3031-3039.

36. C. Ricci, S.G. Kazarian, Collection and detection of latent fingermarks contaminated with cosmetics on nonporous and porous surfaces. *Surf. Interface Anal*. 42 (5) (2010) 386-392.

37. L. Ferguson, F. Wulfert, R. Wolstenholme, Direct detection of peptides and small proteins in fingermarks and determination of sex by MALDI mass spectrometry profiling. *Analyst. 137 (2012) 4686 - 4692*

CHAPTER 5. PAPER TWO

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The effect of moderate temperatures on latent fingerprint chemistry

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5.1 Abstract

The effect of moderate temperatures (25ºC - 75ºC) on latent fingerprints over a 5-hour period was examined using Fourier Transform Infrared (FTIR) microspectroscopy. The aim of the study was to detect changes in IR spectra due to any changes in fingerprint chemistry, these results were then compared to pure compounds found in sebum which were subjected to 75ºC for 5 hours. Latent fingerprints deposited on CaF_2 microscope slides and placed on a Peltier pump heating stage showed that higher temperatures significantly reduced the quantity of sebaceous compounds after 5 hours, whereas temperatures below 45ºC had little effect on the quantity of these compounds over the same time period. FTIR microspectroscopy allowed for the real time detection of changes to the IR spectra and demonstrated an increase in the OH stretch band (3250 cm^{-1}) over 5 hours at all temperatures investigated, suggesting various oxidation processes were taking place. Pure samples analysed included squalene, fatty acids, wax esters and mixed triglycerides. Unsaturated lipids showed a similar increase in the OH stretch band to the latent fingerprints whereas saturated compounds showed no change over time. This information is required to better understand the effect of moderate temperatures on latent fingerprints and how these temperatures could affect aged print composition.

Keywords

Fingerprints, Fourier transform infrared microspectroscopy, aging, thermal degradation, decomposition, oxidation

5.2 Introduction

The chemical degradation of latent fingerprints post-deposition is of great interest to the forensic community. It is well documented that the chemistry of latent fingermarks changes temporally in both adults and children [1-4]. These changes not only affect visualisation techniques [5-7], some being more effective than others on aged fingermarks, but also may potentially provide the basis for an aging tool to establish time since deposition.

The chemical components of a latent fingerprint have been well documented using a variety of analytical techniques [8, 9] but the chemical changes that occur over time are less well understood. Previous studies have shown that there are changes in fingerprint chemistry over time and under different conditions. Temporal decomposition [10-15] appears to involve the shortening and degradation of unsaturated lipids including fatty acids, wax esters, triglycerides, and squalene due to various oxidation processes [16-20]. In contrast, saturated lipids stay relatively stable over longer time periods (>60 days) [20]. A change in unsaturated fatty acids has been observed over time, decreasing significantly over a thirty-day period [12, 20]. This is due to the unsaturated moiety degrading through both aerobic and anaerobic processes and increasing the proportion of saturated compounds [20].

Exposure to light also has a significant impact on fingerprint composition. Studies have shown that exposure to light affects the breakdown mechanisms of various components within fingerprint deposits differently and more rapidly than in dark conditions. Short chain fatty acids and squalene in particular were affected by exposure to light conditions [12]. Squalene (SQ) degradation is supported by the identification of various photo-oxidation mechanisms that produce intermediary products including peroxides, hydroperoxides and SQ epoxide and the fully oxidised forms being hexanedioic and pentanedioic acid [19, 20]. These products are particularly prevalent when squalene is exposed to UV radiation, such as direct sunlight [19-21].

Studies into the temporal changes within latent fingerprints, and exposure to light & dark conditions over time are numerous and have indicated the complex nature of latent fingerprint chemistry. Studies into the effects of other environmental conditions on

fingerprint chemistry, such as temperature, are also prevalent but these pyrolytic degradation studies mostly examine the effect of extreme temperatures (>150^{o}C) from exposure to fires, Improvised Explosive Device (IED) detonations or spent ammunition cartridges [6, 7, 22-25]. Studies into the effects of more moderate temperature variations on latent prints (from room temperature to 80^{o}C) are more limited but did demonstrate a general decrease in lipid components [26]. In contrast to previous studies, detailed examination of moderate temperature effects (25^{o}C -75^{o}C) could provide significant information into the natural aging processes that occur within latent fingerprints.

Fourier transform infrared (FTIR) spectromicroscopy is becoming a recognised tool for forensic research applications. Previous studies have utilised IR techniques to successfully identify the key components of latent fingerprints [10, 15, 26–29], and FTIR is ideally suited to the analysis of latent prints due to its non-destructive nature and specificity in identifying functional groups in organic compounds. FTIR microspectroscopy has proven to be a powerful tool in monitoring temporal changes in latent fingerprints as specific sample areas can be repeatedly analysed over the duration of an experiment [26, 27].

The aim of this study was to investigate the immediate effects of elevated temperatures (25^{o}C to 75^{o}C) on the organic compounds within latent fingerprints from 0 to 5 hours since deposition. Given the known oxidation processes that occur post-deposition of a latent print, this study also aimed to analyse any changes in the IR spectra that could allude to these processes taking place within the first few hours post-deposition. The work was strengthened through the examination of temperature effects on individual chemical components found within latent fingerprints thus enabling a more robust interpretation of observed changes. The compounds selected were either the most abundant in their group, e.g. serine shown to be the most abundant amino acid [30-32, 37], or known to have a significant effect on fingerprint degradation, i.e. squalene [16-21]. The individual components investigated were the steroid precursor squalene, the fatty acids palmitic acid (saturated), and linoleic acid (polyunsaturated), the amino acid serine, the ester myristyl myristate, and mixed saturated and unsaturated triglycerides. These compounds were selected to crudely represent the major components within a latent fingerprint and

as a representative for each of the essential functional groups under investigation in this study.

5.3 Experimental

5.3.1 Sample preparation

All sample fingerprints were obtained from a single donor (a single donor was used for this small scale study to maintain a relative consistency between samples, thus any notable observations were likely to be from the variations in temperature and not simply the inter-variability between fingerprints from multiple donors). Hands were first washed and dried thoroughly. The index finger was then drawn from the bridge of the nose, under the eye to the temple ten times to collect sebaceous secretions and to simulate natural grooming behaviour, and placed directly onto a CaF_2 Infrared (IR) microscope slide (10mm x 10mm, Crystran Ltd). All prints were deposited between 9am and 10am to avoid significant diurnal variations in composition of the latent prints. For each print the same index finger was used. All fingerprints were destroyed at the end of each day and the process repeated for each additional print, this ensured prints were fresh and analysis was not of inadvertently aged prints.

5.3.2 Heating of fingerprints

Once the prints were deposited onto the CaF_2 slides they were placed onto a hollowed out Peltier pump heat stage and placed under the FTIR microscope. The temperatures investigated were 25^{o}C, 35^{o}C, 45^{o}C, 55^{o}C, 65^{o}C, and 75^{o}C. Five prints were analysed at each temperature, five deposits were selected for analysis per print, and spectra were acquired every hour for five hours at relative humidity.

5.3.3 FTIR microspectroscopy

The latent fingerprints were analysed using a PerkinElmer Spectrum^{TM} Spotlight 200 FTIR imaging System equipped with a liquid-nitrogen cooled MCT linear array detector. Data was analysed using Perkin Elmer software, Spectrum® (v10.02.00), variations in spectra were processed using peak area calculations. Spectra from fingerprint deposits were collected in transmission mode, the IR beam passing through the slide and the hollowed centre of the heat stage, within 4000 to 750 cm^{-1} spectral range with 10 scans per pixel at 4 cm^{-1} spectral resolution and 10 µm spatial resolution, using a 100 x 100 µm aperture.

5.3.4 Pure sample preparation

Squalene ≥98% (Sigma Aldrich, UK), linoleic acid ≥99% (Sigma Aldrich, UK), serine ≥99% (Sigma Aldrich, UK), myristyl myristate ≥99% (Sigma Aldrich, UK) and mixed saturated and unsaturated triglycerides ≥98% (100 mg triacetin, tributyrin, tricaproin, tricaprylin, tricaprin, all equal amounts by weight) (Sigma Aldrich, UK). 10 µL of the pure compound was pipetted onto a CaF_2 slide and analysed in an identical way to the latent fingerprints at 75^{o}C for 5 hours. Analysis at this temperature allowed for a definitive indication as to whether these compounds were susceptible to thermal degradation.

5.4 Results and Discussion

Before any fingerprints were subjected to heat treatment a control spectrum was obtained from each print. Figure 24 shows a typical FTIR spectrum of a fresh fingerprint from this study, the key peaks ranging from 3000 cm^{-1} to 1100 cm^{-1}.

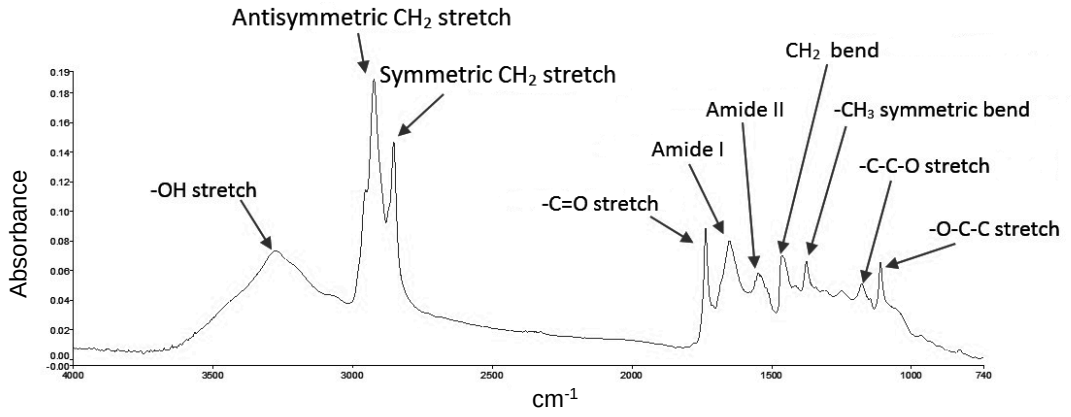

Figure 24: A typical FT-IR spectrum of a fresh fingerprint from the study subject

This study predominantly focused on variations in quantity of sebaceous materials at different temperatures. These sebaceous compounds correspond to the 2920 cm^{-1} and 2854 cm^{-1} antisymmetric and symmetric C-H stretching modes of CH_2 groups, the C=O stretch of lipids (1741 cm^{-1}), the scissoring mode of CH_2 groups and antisymmetric C-H bending mode of CH_3 groups (1463 cm^{-1}), the CH_3 symmetric bend (1380 cm^{-1}), the C-C-O stretch (1160 cm^{-1}), and the O-C-C stretch (1111 cm^{-1}). Variations in quantity of eccrine secretions were also analysed, amide bonds of peptides and proteins were observed corresponding to the amide I band (1655 cm^{-1}), and amide II band (1545 cm^{-1}) of secondary amides. The broad O-H stretch band (3250 cm^{-1}) was also observed. It is changes to these sebaceous and eccrine absorption bands that correspond to changes in quantity of the related functional groups and therefore the compounds over time.

5.4.1 Thermally degraded fingerprints

Analysis of fingerprints exposed to moderate temperatures indicates significant changes in composition over time.

This study showed a significant reduction in absorbance, and therefore material, of all the key functional groups over the five hours at 75ºC, 65ºC, 55ºC with the exception of the amide bands, but no significant reduction at the lower temperatures of 45ºC, 35ºC, and 25ºC (figures 25 and 26).

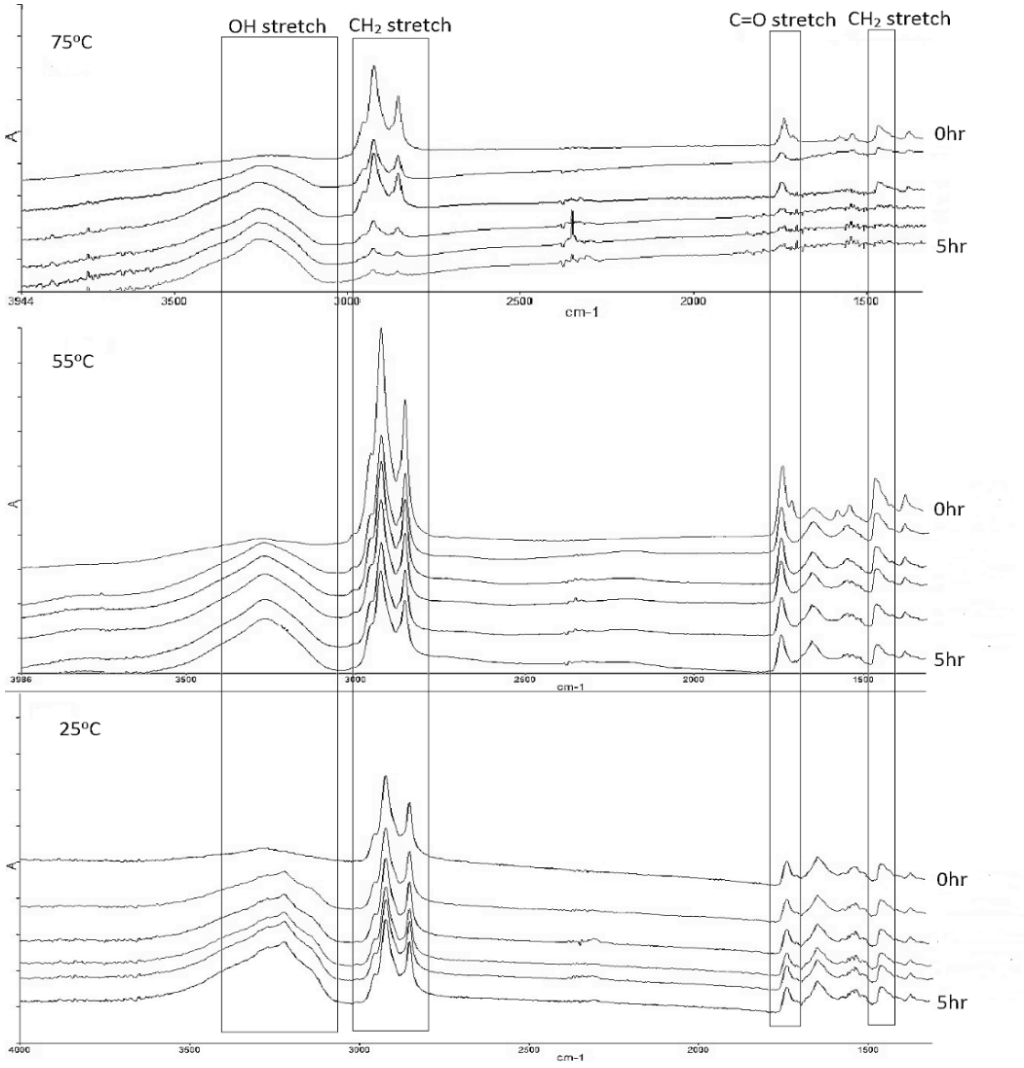

Figure 25: Absorbance spectra of latent fingerprints as a function of time and temperature for 75ºC, 55ºC, and 25ºC.

This suggests that at the higher temperatures the more volatile sebaceous substituents are degrading rapidly, but at the lower temperature (45ºC, 35ºC, 25ºC) there is less degradation over this short time period, certainly at 35ºC and 25ºC there is no observable change in absorbance between the control and after 5 hours (see Appendix A2 for visual comparisons).

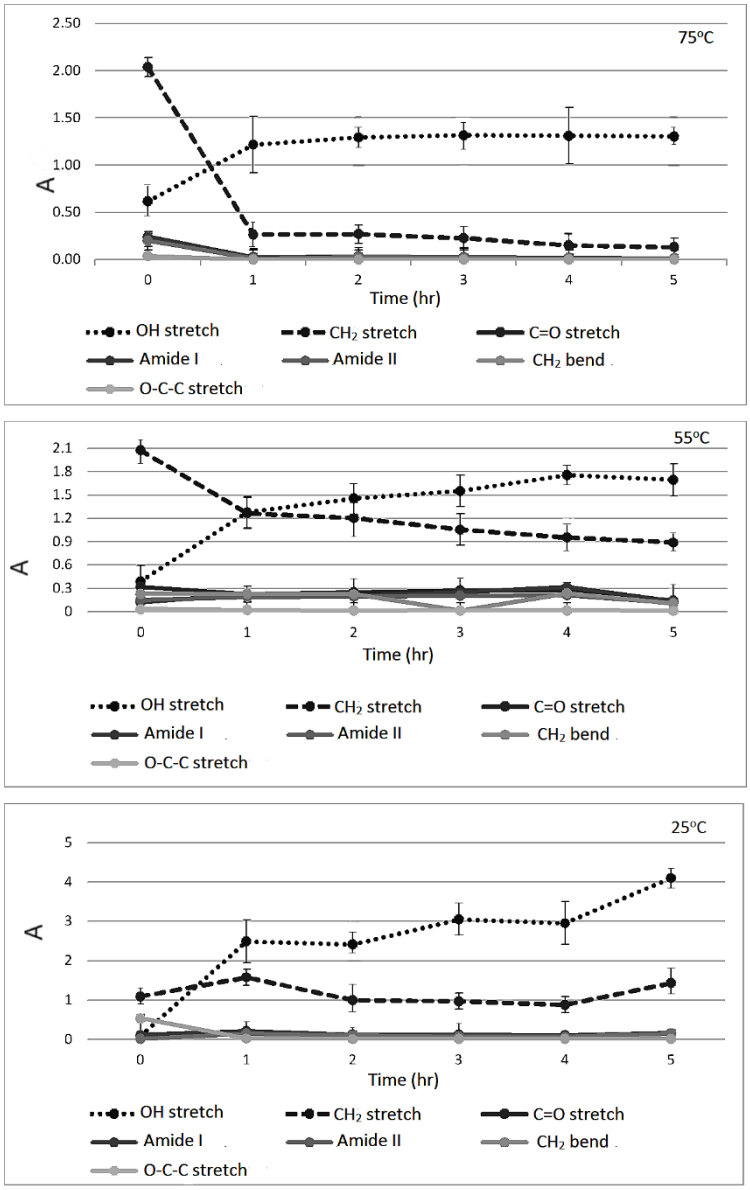

Figure 26: Changes in average IR absorbance of key functional groups over time at 75ºC, 55ºC, and 25ºC. A reduction in material at temperatures at 55ºC and above, with the exception of the OH group which increased. At 45ºC and below there was no reduction in composition over the 5 hours, but the OH stretch region increased at all temperatures (n = 25, error bars represent standard error of the mean)

This is to be expected given the far longer time periods required for temporal degradation as observed in the studies mentioned previously, all being performed at room temperature. The reduction in sebaceous material at higher temperatures is understood to be due to the degradation through oxidation of lower molecular weight, volatile unsaturated lipids, including unsaturated fatty acids, triglycerides as well as squalene [12, 19, 20]. Squalene has been reported to breakdown rapidly in aged fingerprints reducing in quantity within a day and being almost entirely undetectable within one week [19], and this process it likely to be accelerated at elevated temperatures.

Figure 27 shows the rate of change in absorbance for the 2920 cm^{-1} and 2854 cm^{-1} C-H stretching modes of CH_2 groups at all six temperatures.

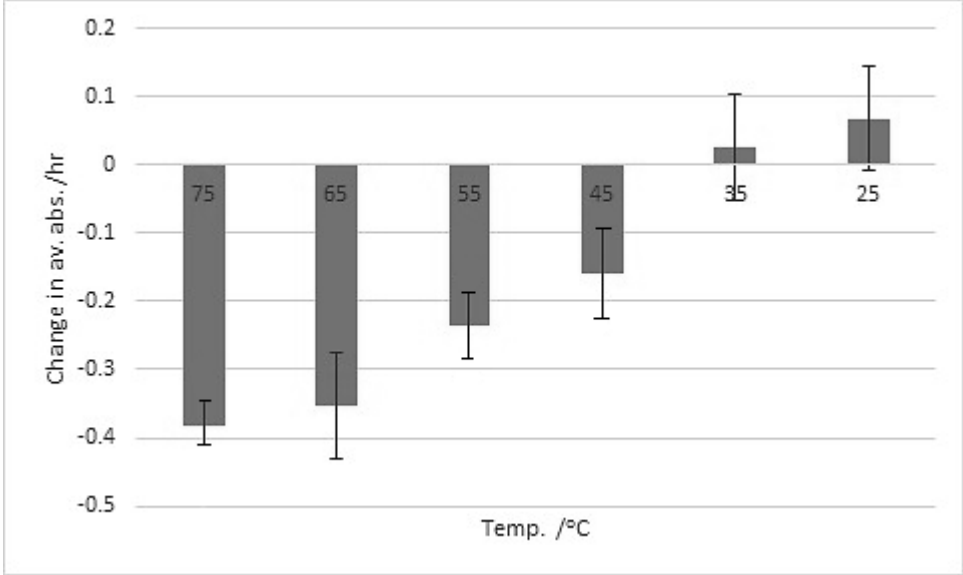

Figure 27: Rate of change of the C-H stretch band absorbance at 75ºC, 65ºC, 55ºC, 45ºC, 35ºC, and 25ºC (n = 25, error bars represent standard error of the mean)

Samples heated to between 45-75ºC show a significant decrease in C-H absorbance meaning a reduction in material, the fastest reduction in absorbance being at the highest temperatures. The slight increase in the C-H stretching band at 35ºC and 25ºC could be an indication of an initial increase in short chain saturated fatty acids as identified in previous studies [12], which at higher temperatures evaporate off due to their volatility.

The lack of change in the amide I and II bands (Figure 26) is unsurprising given previous research demonstrating that these compounds do not seemingly undergo any significant

photo-degradation over time and thermal degradation is only seen with temperatures in excess of 100^{o}C [25].

Most notable in this study was the significant increase in absorbance of the OH stretch band (3250 cm^{-1}) across all temperatures. This is in contrast to previous temporal degradation studies that indicate a decrease in OH absorbance over time [15] thought primarily to be due to a loss of water through evaporation over periods as long as six weeks.

The rapid oxidation of sebaceous compounds such as unsaturated fatty acids, squalene and glycerides could account for the increase of the OH stretch absorption band. Even accounting for water loss, the net gain being the formation of these hydroxide functional groups due to oxidation mechanisms forming acids, alcohols, and hydroperoxides [12, 19, 20]. This data suggests that the activation energies required for these processes are below 25^{o}C as there was no statistical relationship between rate of change for the increase in the OH stretch band over time and temperature. Given the previously reported rapid degradation of sebaceous compounds such as squalene and certain unsaturated fatty acids, it is likely that these processes require a low activation energy for oxidation to occur. Although the temperatures tested in this study do not influence the rate of change of the OH absorption band, higher temperatures (65^{o}C and 75^{o}C) do appear to limit the quantity of hydroxide functional groups being formed when compared to lower temperatures (Figure 26). This is most likely to be due to a more rapid loss of water at these higher temperatures as well as a loss of the more volatile organic compounds.

5.4.2 Pure compounds

If specific sebaceous secretions are responsible for these oxidation processes and the resulting increase in the OH stretch band of the IR spectra, it is important to identify which compounds within these secretions are the main contributors to this change. As mentioned above previous studies have demonstrated that certain unsaturated lipids decompose rapidly and could therefore contribute to the increase in absorbance of the OH stretch band at 3250 cm^{-1}. This study looked at the major components of a latent fingerprint to identify which pure compounds, such as squalene, or compound groups, such as fatty acids, most readily decomposed and contributed to the increase in absorbance of the OH band. Compounds primarily found in sebaceous secretions were selected, namely squalene, palmitic acid (saturated fatty acid), linoleic acid (polyunsaturated fatty acid), myristyl myristate (wax ester), and mixed triglycerides, as well as the amino acid serine, an eccrine secretion. Figure 28 shows example spectra of the changes in the OH stretch band over time for squalene.

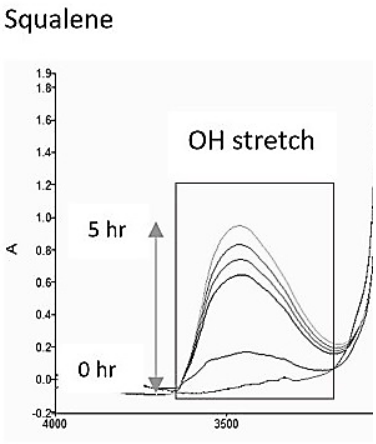

Figure 28: Infrared spectra of the 3250 cm^{-1} OH-stretch band of pure squalene heated to 75ºC for 5 hr

Of the six compound groups investigated in this study (Figure 29), squalene, the mixed triglycerides and linoleic acid showed significant changes in the OH stretch region at 3250 cm^{-1}. Serine, palmitic acid and myristyl myristate showed no significant changes over the duration of the experiment. This data suggests that thermal degradation processes impact unsaturated sebaceous compounds significantly, but not saturated sebaceous compounds or amino acids.

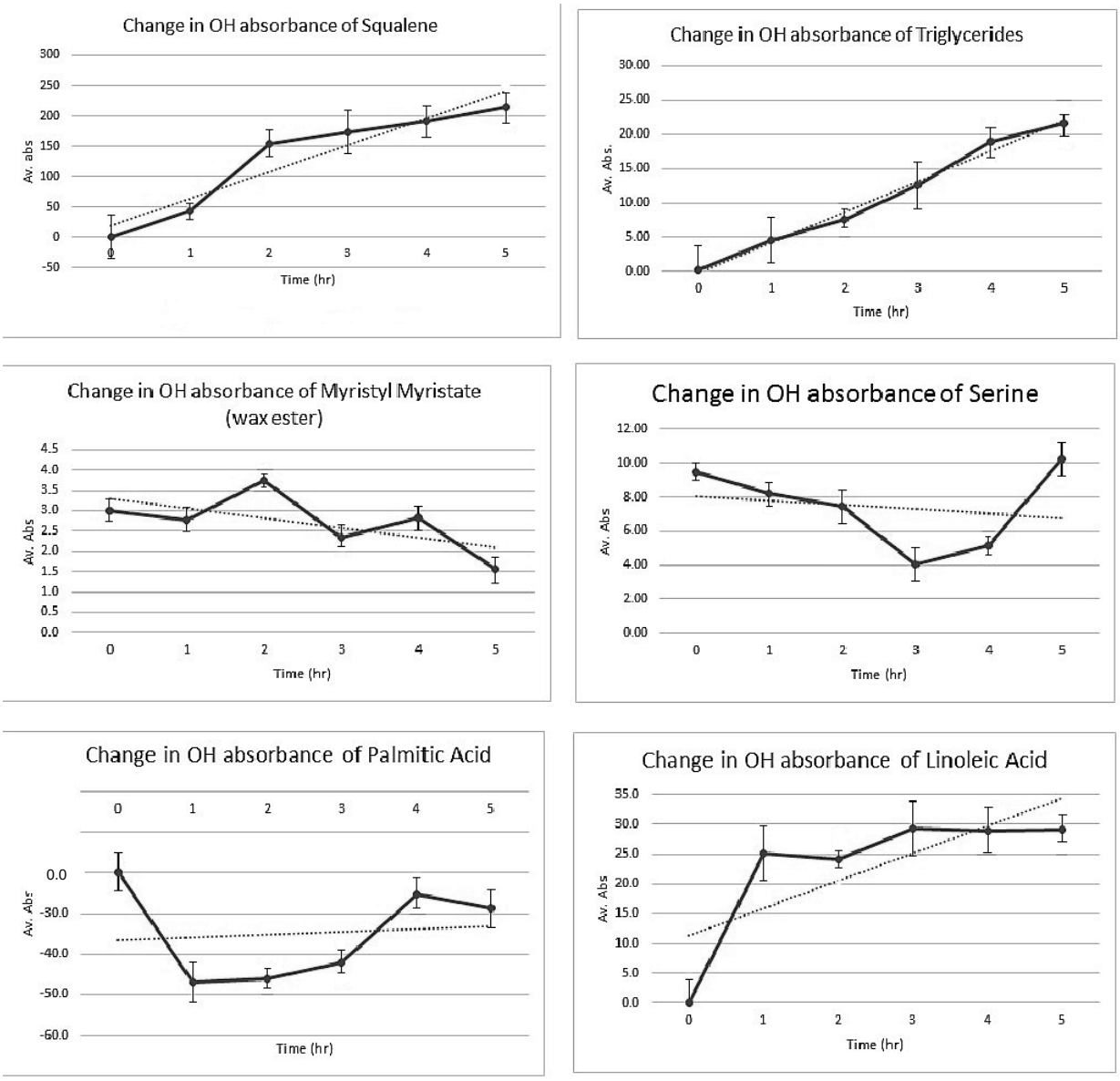

Figure 29: The increase in OH absorbance band (3250 cm^{-1}) of SQ, mixed triglycerides, myristyl myristate, serine, palmitic acid, and linoleic acid at 75ºC over 5h, demonstrating oxidation processes taking place in unsaturated compounds (n = 20, error bars represent standard error of the mean)

The increase in the OH stretch region at 3250 cm^{-1} of squalene supports previous studies reporting the rapid oxidation of this compound to produce intermediaries such as alcohols, mono- and polyhydroperoxides, epoxides and finally hexanedioic acid and pentanedioic acid. The mixed triglycerides analysed showed an increase in the OH stretch band over the 5 hours, indicating degradation processes in action, although not to the same extent as squalene (Figure 29). Some previous research has suggested possible triglyceride degradation mechanisms resulting in both saturated and unsaturated fatty acids [33, 34], the unsaturated fatty acids then likely go through further oxidation thus continuing to increase the OH band. In a latent fingerprint it is likely that triglyceride degradation processes would be increased due to the presence of cholesterol which has been shown to affect triglyceride decomposition [34]. Of the fatty acids (FA) analysed, linoleic acid (Figure 29), a polyunsaturated FA, showed a rapid initial increase in the OH stretch region in the first hour, then little change over the remaining four hours. This suggests rapid oxidation occurring on this unsaturated compound perhaps, as previously proposed, to produce fatty acid peroxides through aerobic degradation [20, 35]. It is worth remembering however that polyunsaturated FA's only make up ~2% of sebaceous secretions [17, 36] so it is unlikely that these compounds would have a significant effect on the increase of the OH stretch band of a latent fingerprint. Monounsaturated FA's would also degrade aerobically however and they make up ~48% of sebum [17, 36]. The OH stretch band of palmitic acid, a saturated fatty acid, showed no significant variation in this study, this is consistent with temporal degradation studies reporting that C16 FA's such as palmitic acid remain relatively stable over a 60-day period [20]. The OH stretch band of myristyl myristate also showed no significant changes over the duration of this study, again consistent with previous studies showing that these saturated lipids are not significantly affected by temporal degradation due to a lack of functional groups prone to degradation [17]. Serine, the only non-sebaceous compound analysed in this study showed no correlation between temperature and changes in the OH stretch region. As mentioned above previous studies have shown that significant thermal degradation of amino acids occurs at 100^{o}C and higher [24, 25] due to their low volatility, so it is not surprising that there were no significant changes in the 3250 cm^{-1} OH stretch band in this study.

5.5 Conclusions

The research presented within this paper suggests that the oxidation processes that occur during thermal degradation of latent fingerprints could be similar if not identical to that of temporal aging at a constant ambient temperature as shown in previous studies [12, 16, 19, 20], but at a faster rate. It is reasonable to assume that latent fingerprints at a crime scene would be subject to varying diurnal temperatures from the time of deposition to the time of analysis by law enforcement agencies, varying from hours to days. This study demonstrates the dynamic nature of chemical decomposition within a fingerprint after just a few hours, higher temperatures causing more rapid degradation of the unsaturated and volatile lipids, which could impact crime scene work. Developing fingerprints that have been exposed to higher diurnal temperatures could be more challenging, particularly when using those developing agents that rely on lipophilic interactions. Further work is required to investigate the extent and consistency of these degradation processes using multiple donors to identify trends in decomposition, as well as the effect of different substrates on fingerprint chemistry. Future studies to better understand the degradation products that are likely to occur at varying temperatures could lead to better development techniques, more suited to prints subjected to higher diurnal temperatures.

The pure materials analysed in this study translate to the compounds involved in the degradation processes of natural fingerprints. The analysis of pure samples that are present in sebum showed that squalene in particular, but also triglycerides and mono/poly-unsaturated fatty acids, are most likely to be the cause of an increase in the OH stretch band at 3250 cm^{-1} over time, this is likely to be due to various intermediary and complete oxidation processes. Future work accounting for individual variability would provide a better understanding of the interactions between these compounds and how each component affects the stability of the other.

5.6 References

1. M.V. Buchanan, K. Asano, A.I. Bohanon. Chemical characterization of fingerprints. *SPIE, Forensic Evidenc Anal Crime Scene Investig*. 1997. 2941:89-95.

2. D.K. Williams, C.J. Brown, J. Bruker. Characterization of children's latent fingerprint residues by infrared microspectroscopy: Forensic implications. *Forensic Sci Int*. 2011. 206(1-3):161-165.

3. D. Noble. Vanished into thin air: The search for children's fingerprints. *Anal Chem*. 1995. 67(13):435A-438A.

4. K.M. Antoine, S. Mortazavi, L.M. Miller. Chemical differences are observed in children's versus adults' latent fingerprints as a function of time. *J. Forensic Sci*. 2010. 55(2):513-518.

5. K. Sampson, W. Sampson. Recovery of Latent Prints from Human Skin. *J. Forensic Identif*. 2005. 55(3):362-385.

6. A.G. Brown, D. Sommerville, B.J Reedy, R.G Shimmon, M. Tahtouh. Revisiting the thermal development of latent fingerprints on porous surfaces: new aspects and refinements. *J. Forensic Sci*. 2009. 54(1):114-121.

7. A. Dominick, N. Nicdaeid, S. Bleay, V. Sears. The recoverability of fingerprints on paper exposed to elevated temperatures -Part 1: comparison of enhancement techniques. *J. Forensic Identif*. 2010. 59(3):325-339.

8. S. Cadd, M. Islam, P. Manson, S. Bleay. Fingerprint composition and aging: A literature review. *Sci Justice*. 2015. 55(4):219-238.

9. M.J. Bailey, N.J. Bright, S. Hinder et al. Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spec. *Anal Chem*. 2012. 84(20):8514-8523.

10. A. Girod, L. Xiao, B. Reedy, C. Roux, C. Weyermann. Fingermark initial composition and aging using Fourier transform infrared microspectroscopy (μ-FTIR). *Forensic Sci Int*. 2015. 254:185-196.

11. F. Cuthbertson, J.R. Morris. *The Chemistry of Fingerprints,* AWRE Report No. 013/69, Atomic Energy Authority, U.K., 1969.

12. N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells. Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface. *Forensic Sci Int*. 2005. 154(2-3):224-239.

13. R.S Croxton, M.G. Baron, D. Butler, T. Kent, V.G Sears. Variation in amino acid and lipid composition of latent fingerprints. *Forensic Sci Int*. 2010. 199:93-102.

14. A. Girod, R. Ramotowski, C. Weyermann. Composition of fingermark residue: A qualitative and quantitative review. *Forensic Sci Int*. 2012. 223(1-3):10-24.

15. P. Fritz, W. van Bronswjik, K. Lepkova et al. Infrared microspectroscopy studies of the chemical composition of latent fingermark residues. *Microchem J*. 2013. 111:40-46.

16. C. Weyermann, C. Roux, C. Champod. Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis. *J. Forensic Sci*. 2011. 56(1):102-108.

17. R. Ramotowski. *Composition of Latent Print Residues, in: Advanced in Fingerprint Technology, 2nd Ed.* (Lee. H, Gaensslen. R, eds.). London: CRC Press. 2001.

18. R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese. Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids. *Rapid Commun Mass Spectrom*. 2009. 23(19):3031-3039.

19. K.A. Mountfort, H. Bronstein, N Archer, S.M. Jickells. Identification of oxidation products of squalene in solution and in latent fingerprints by ESI-MS and LC/APCI-MS. *Anal Chem*. 2007. 79(7):2650-2657.

20. G.M. Mong, C.E. Petersen, T. Clauss. *Advanced Fingerprint Analysis Project - Fingerprint Constituents*. Vol PNNL-13019. Pacific Northwest National Laboratory: U.S. Department of Energy. 1999.

21. M. Picardo, C. Zompetta, C. De Luca et al. Squalene peroxides may contribute to ultraviolet light-induced immunological effects. *Photodermatol Photoimmunol Photomed*. 1991. 8(3):105-110.

22. C.M. Girelli, B.J.M. Lobo, A.G. Cunha, J.C.C. Freitas, F.G. Emmerich. Comparison of practical techniques to develop latent fingermarks on fired and unfired cartridge cases. *Forensic Sci Int*. 2015. 250:17-26.

23. W.W. Harper. Latent Fingerprints at High Temperatures. *J. Crim Law Criminol*. 1938. 29(4):580-583.

24. A. Richmond-Aylor, S. Bell, P. Callery, K. Morris. Thermal degradation analysis of amino acids in fingerprint residue by pyrolysis GC-MS to develop new latent fingerprint developing reagents. *J. Forensic Sci*. 2007. 52(2):380-382.

25. G. De Paoli, S.A. Lewis, E.L. Schuette, L.A. Lewis, R.M. Connatser, T. Farkas. Photo- and thermal-degradation studies of select eccrine fingerprint constituents. *J. Forensic Sci*. 2010. 55(4):962-969.

26. C. Ricci, P. Phiriyavityopas, N. Curum, K.L. Andrew Chan, S. Jickells, S. Kazarian. Chemical Imaging of Latent Fingerprint Residues. *Appl Spectrosc*. 2007. 61(5):514-522.

27. D.K. Williams, R.L. Schwartz, E.G. Bartick. Analysis of Latent Fingerprints Deposits by Infrared Microspectroscopy. *Appl Spectrosc*. 2004;58(3):313-316.

28. N.J. Crane, E.G. Bartick, R.S. Perlman, S. Huffman. Infrared spectroscopic imaging for noninvasive detection of latent fingerprints. *J Forensic Sci*. 2007;52(1):48-53.

29. A. Hemmila, J. McGill, D. Ritter. Fourier transform infrared reflectance spectra of latent fingerprints: a biometric gauge for the age of an individual. *J. Forensic Sci*. 2008;53(2):369-376.

30. B. Hadorn, F. Hanimann, P. Anders, H. Curtius, R. Halverson. Free Amino-acids in Human Sweat from Different Parts of the Body. *Nature*. 1965. 215(99):416-417.

31. P. Hamilton. Amino-acids on Hands. *Nature*. 1965. 205:284-285.

32. J. Oro, H. Skewes. Free Amino-Acids on Human Fingers: The Question of Contamination in Microanalysis. *Nature*. 1965. 207(1):1042-1045.

33. A. Srivastava, R. Prasad. Triglycerides-based diesel fuels. *Renew Sust Energy Rev*. 2000. 4(2):111-113.

34. L. Iuliano. Pathways of cholesterol oxidation via non-enzymatic mechanisms. *Chem Phys Lipids*. 2011. 164(6):457-468.

35. B.B. Dent, S.L. Forbes, B.H. Stuart. Review of human decomposition processes in soil. *Environ Geol*. 2004. 45(4):576-585.

36. A. Girod, C. Weyermann. Lipid composition of fingermark residue and donor classification using GC/MS. *Forensic Sci Int*. 2014. 238:68-82.

37. T. Atherton, R. Croxton, M. Baron, J. Gonzalez-Rodriguez, L. Gámiz-Gracia, A.M. García-Campańa. Analysis of amino acids in latent fingerprint residue by capillary electrophoresis-mass spectrometry. *J. Sep Sci*. 2012. 35:2994-2999.

CHAPTER 6. PAPER THREE

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A study of the intermolecular interactions of lipid components from analogue fingermark residues.

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6.1 Abstract

A compositionally simplified analogue of a latent fingermark was created by combining single representatives of each major component of a natural fingermark. Further modified analogues were also produced each having one component removed. The aim of this study was to investigate the intermolecular interactions that occurred within these analogue samples using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectroscopy showed that the absence of squalene and cholesterol significantly restricted the interactions between the other organic constituents within the analogue samples. Investigating the intermolecular interactions of organic compounds within a simplified analogue solution could indicate corresponding interactions that occur within natural fingermarks. These potential interactions could go on to be the target of further investigation of latent fingermark chemistry, and ultimately contribute to a better understanding of the aging processes and degradation mechanisms that take place post-deposition.

Keywords

Latent fingermarks, analogue, lipids, decomposition, squalene, chlolesterol.

6.2 Introduction

The dynamic nature of fingermark chemistry post-deposition is a complex process, yet of great interest to the forensic community. Numerous studies have examined the aging of fingermarks, the oxidation mechanisms that take place, both short and long term [1, 2], and the resulting end products [2-5], [8-12]. However, an in-depth understanding of fingermark degradation processes remains limited. Various studies have investigated the breakdown of key components within a fingermark such as squalene [1–3], [9], fatty acids [2-5, 7], cholesterol [4], [9–11], [14], and amino acids [13,15,16], although very little research has studied the intermolecular interactions between these constituents and their impact on each other. Ultimately, a better understanding of these intermolecular interactions within a fingermark will allow for improved modelling of the fingermark aging process.

Cholesterol and its oxidation products have a significant effect on the decomposition of triglycerides and fatty acids [10], indicating that lipid stability is influenced by these intermolecular interactions within latent fingermarks. Auto-oxidation, oxidation in the absence of enzymatic catalysis, of cholesterol by free radicals and hydroperoxides to form oxysterols is an established degradation mechanism [10], although evidence of these oxidation products are yet to be found in latent fingermarks. It has also been shown that the decomposition of cholesterol can be accelerated by both triglycerides and fatty acids [11], [17], [18]. This suggests a form of positive feedback wherein cholesterol affects the decomposition of triglycerides resulting in a mixture of saturated and unsaturated fatty acids [6]. This increase in fatty acid concentration could then increase the rate of decomposition of cholesterol.

Squalene is an unsaturated steroid precursor that has received a great deal of attention in previous studies of fingermark degradation mechanisms and the aging of fingermarks [1–3], [9], [12]. In latent fingermarks squalene degrades rapidly over time through direct oxidation and photo-oxidation mechanisms. Measurable reductions in concentration can be demonstrated within 24 hours following deposition and it is almost undetectable after a week [1]. Although this degradation is dependent on light conditions as squalene can still be detected in fingermarks up to 33 days after deposition when stored in the dark [3].

During degradation various intermediary and complete oxidation products have previously been identified [1, 2]. Of particular note is the formation of various hydroperoxides as squalene decomposes through direct oxidation. These hydroperoxides then undergo thermolysis with homolytic scission of the peroxide bond, yielding hydroxyl radicals [18]. Both hydroperoxides and free radicals, such as hydroxyl radicals produced from squalene degradation, would then impact cholesterol breakdown and impact the degradation of triglycerides and fatty acids.

This investigation utilised Fourier transform infrared (FTIR) microspectroscopy, a recognised tool for forensic research applications. Previous studies using IR techniques have successfully identified the key components of latent fingermarks [19-25]. FTIR spectroscopy is ideally suited to the analysis of latent fingermarks due to its non-destructive nature and specificity in identifying functional groups in organic compounds. Of particular relevance to this study FTIR spectroscopy lends itself to studying the intermolecular interactions within fingermarks as a major contributor to IR band width is the strength of intermolecular interactions. Broader peaks being a function of stronger intermolecular interactions over a wider range and therefore a large number of energy states.

The antisymmetric and symmetric C-H stretch of the CH_2 groups at 2920 – 2850 cm^{-1} within a fingermark are not truly decoupled due to the slight variation in chemical environment of the differing species present. Intermolecular forces such as hydrogen bonding, dipole/dipole interactions, and London Dispersive Forces all combine to increase organic molecular interactions [26-32]. These interactions will dictate the rate of reaction and therefore the molecular decomposition in the presence of (in particular) long chain unsaturated species. These intermolecular interactions affect the range of vibrational modes of the C-H stretch region of CH_2 groups, increasing variations in bond distance and changing the spring constant [31-35]. The more intermolecular interactions occurring the broader the antisymmetric and symmetric vibrational modes and the coupling effect visible on the spectrum (figure 30). Fewer intermolecular interactions perturb the range of vibrational modes of the C-H stretch band at 2920 – 2850 cm^{-1},

minimising the coupling effect of the antisymmetric and symmetric C-H peaks, therefore producing two distinct peaks at 2920 – 2850 cm^{-1}.

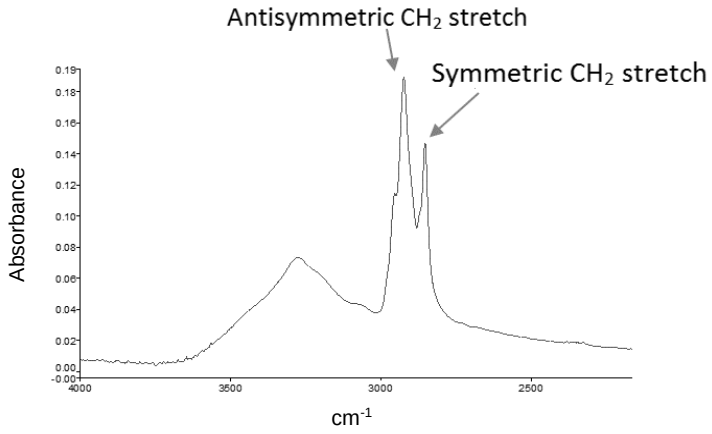

Figure 30: Coupling effect of the antisymmetric and symmetric C-H stretch of CH_2 group

The aim of this study was to generate a simplified chemical analogue of a fingermark, comprised of a single representative of the major components, in order to better understand the interactions between these compounds. Studying these intermolecular interactions could ultimately enable a better understanding of their effect on the aging of fingermarks post-deposition. Once an acceptable analogue was developed and the IR spectra compared to that of sebaceous-loaded fingermarks, further analogues were developed, each with one component removed, and IR spectra were then obtained. This allowed assessment of how this removed component affected the interactions of the others.

Replicates of latent fingermarks have previously been developed for both research and commercial purposes [5, 36, 37] to provide a ‘standardised’ deposition model, and with more complexity than the analogue samples developed for this study. The objective of this study however was not to create a complete replicate of a latent fingermark, but to deliberately develop a much simplified composition that allowed for fundamental analysis of any critical intermolecular interactions that may occur. More complex replicates involving hundreds of compounds would have made analysis of specific intermolecular

interactions near impossible, or at least far less conclusive. Another reason why such a simplified composition was used in this study was because, as stated previously [37], synthetic solutions can behave differently to natural fingermarks, and the more complex the solution the more potential there is for inconclusive variability in the results.

A primary use for replicate solutions is for identifying effective fingermark development reagents. The International Fingerprint Research Group (IFRG) have stated that replicates are useful for fundamental research and initial study of molecular interactions, but are not appropriate for optimisation or validation trials [36], and have warned caution about using (particularly) synthetic lipid solutions for direct evaluation with natural latent fingermarks, although this was specifically regarding fingermark development reagents [38].

It is evident therefore that a degree of caution must be employed when using a simplified analogue solution to study the chemical interactions within fingermarks, and certainly no direct comparisons between the two can be made. This study therefore aimed to provide a general indication of the intermolecular interactions that *may* occur within latent fingermarks, and thus present a target for further work to look for these potential interactions in latent fingermarks and their impact on fingermark degradation.

6.3 Experimental

6.3.1 Sample preparation

6.3.1.1 Analogue ‘fingermark’ preparation

The composition of the analogue solution was based on the principal compounds observed during previous studies of latent fingermarks [2], [5], [19], [37], [39], [40]. Where there was significant variation in the literature regarding the concentration of a component, an average of all available data was used.

Sebaceous and organic eccrine secretions were selected for the analogue. The sebaceous secretions comprised a sterol, sterol precursor, a fatty acid, mixed triglycerides (100 mg triacetin, tributyrin, tricaproin, tricaprylin, tricaprin, all equal amounts by weight), and a wax ester. The eccrine secretions were composed from an amino acid and lactic acid. For simplicity, the most abundant compound within each family (i.e. amino acid, fatty acid, wax ester) was selected to represent the compounds within that family. For example, serine, being the most abundant amino acid [41–43] was selected to represent all amino acids, and palmitic acid, the most abundant fatty acid, represented all fatty acids [2, 3] (Unsaturated fatty acids, although more likely to have an impact on intermolecular interactions due to a targetable functional group [40], were not included in this study due to lack of data on origin or quantity).

This abundancy approach was also reflected in the concentrations of each compound that made up the analogue solution, which represented the total concentration of that compound group within a latent fingermark. For example, the concentration of serine in the analogue was 1.45 mgL^{-1}, equivalent to the typical total concentration of amino acids observed in eccrine sweat [5, 12]. The compounds selected and their concentrations are shown in table 6 (all compounds ≥98%, obtained from Sigma Aldrich, UK).

Compound	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	myristyl myristate
Concentration	11.2 mg/L^{-1}	1.1 mg/L^{-1}	15.4 mg/L^{-1}	2.5 mg/L^{-1}	10 mg/L^{-1}	42.1 mg/L^{-1}	29.9 mg/L^{-1}

Table 6: The compounds and concentrations selected to create the analogue 'fingermark' composition

Table 7 shows the composition of each sample. Each sample was made up to a 1 L solution with distilled water. Sample 1, comprised of all the constituents shown in table 6, was used as the control and samples 2-8 all having one of the compounds in table 6 removed.

Sample 1 'complete'	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 2	-	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 3	Squalene	-	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 4	Squalene	Cholesterol	-	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 5	Squalene	Cholesterol	Palmitic acid	-	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 6	Squalene	Cholesterol	Palmitic acid	Serine	-	Mixed triglycerides	Myristyl myristate
Sample 7	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	-	Myristyl myristate
Sample 8	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	-

Table 7: The composition of the eight analogue 'fingermark' samples

During formulation each sample was heated to 37^{o}C to replicate body temperature and to force any solid compounds into solution with the addition of 50 mgL^{-1} of the emulsifier oleyl alcohol. Immediately after mixing 10 µL of the analogue samples were pipetted onto CaF_2 Infrared (IR) microscope slides (10 mm x 10 mm, Crystran Ltd) and placed on a Peltier pump heat stage to maintain 'body temperature' and placed under the IR microscope for analysis. Each sample was analysed 20 times and an average spectrum obtained.

6.3.1.2 Sebaceous-loaded fingermarks

All fingermarks were obtained from a single donor (38-year-old male, wearing no cosmetics) to maintain a relative consistency between samples, and the spectra obtained were consistent with data from previous studies. Hands were first washed and dried thoroughly. The index finger was then drawn from the bridge of the nose, under the eye to the temple ten times to collect sebaceous secretions and to simulate natural grooming behaviour, and placed directly onto a CaF_2 IR microscope slide (10mm x 10mm, Crystran Ltd). All fingermarks were deposited between 9am and 10am to avoid significant diurnal variations in composition of the latent fingermark and analysed immediately. For each fingermark the same index finger was used. 20 samples were analysed at room temperature and pressure and an average spectrum obtained from 10 randomly chosen locations per fingermark.

6.3.2 FTIR Microspectroscopy

The sebaceous-loaded fingermarks and the analogue compositions were analysed using a PerkinElmer Spectrum™ Spotlight 200 FTIR imaging System equipped with a liquid-nitrogen cooled MCT linear array detector. Data was analysed using Perkin Elmer software, Spectrum® (v10.02.00), variations in spectra were processed using peak area calculations. Spectra were collected in transmission mode, the IR beam passing through the slide, within 4000 to 750 cm^{-1} spectral range with 10 scans per pixel at 4 cm^{-1} spectral resolution and 10 µm spatial resolution, using a 100 x 100 µm aperture.

6.4 Results and Discussion

Prior to analysis of the analogue samples, spectra from sebaceous-loaded fingermarks were obtained to serve as a spectral comparison to the complete analogue sample. Figure 31 shows a typical FTIR spectrum of a fresh fingermark, the key peaks ranging from 3000 cm^{-1} to 1100 cm^{-1}.

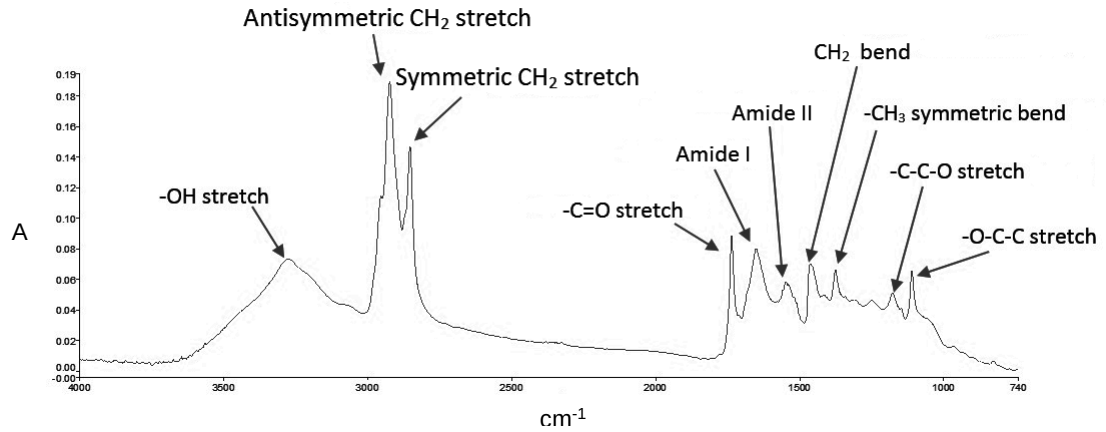

Figure 31: Typical FTIR Spectrum of a natural fingermark

This study predominantly focused on the interactions between sebaceous materials present within fingermarks. These sebaceous compounds correspond to the 2920 cm^{-1} and 2854 cm^{-1} antisymmetric and symmetric C-H stretching modes of CH_2 groups, the C=O stretch of lipids (1741 cm^{-1}), the scissoring mode of CH_2 groups and antisymmetric C-H bending mode of CH_3 groups (1463 cm^{-1}), the CH_3 symmetric bend (1380 cm^{-1}), the C-C-O stretch (1160 cm^{-1}), and the O-C-C stretch (1111 cm^{-1}). Some eccrine secretions were also analysed, amide bonds of peptides and proteins were observed corresponding to the amide I band (1655 cm^{-1}), and amide II band (1545 cm^{-1}) of secondary amides.

6.4.1 Analogue samples

The ‘complete’ analogue sample demonstrated an accurate IR spectral representation of a sebaceous-loaded fingermark (figure 32).

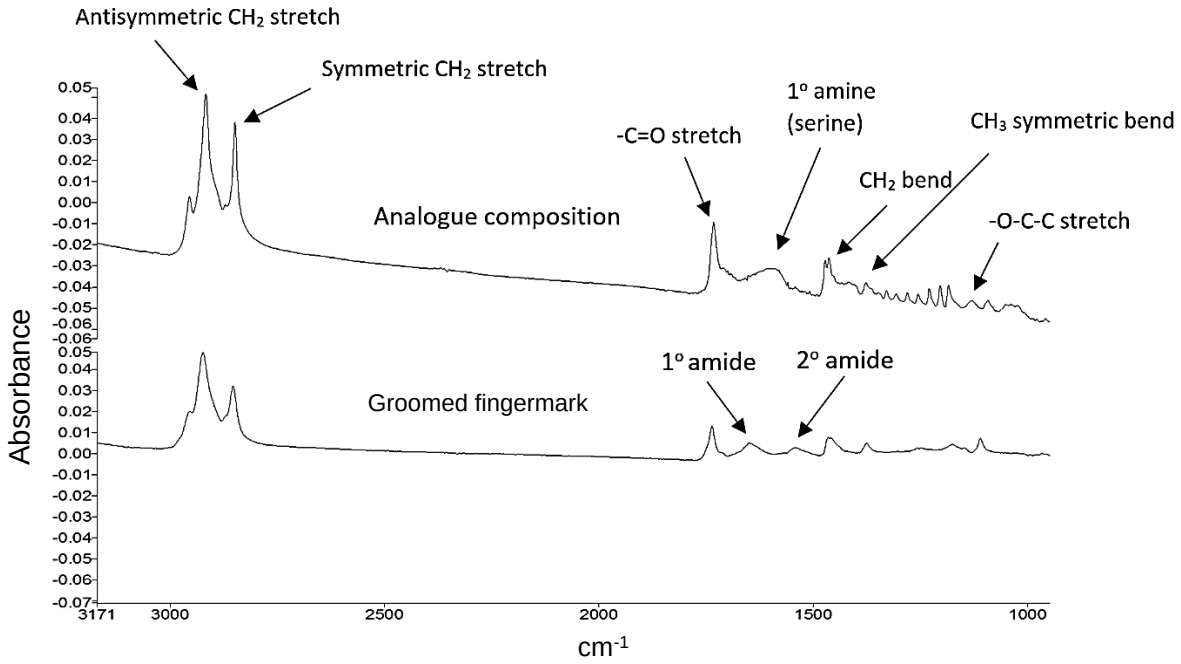

Figure 32: FTIR spectral comparison between the analogue sample and a natural fingermark.

The spectra of the ‘complete’ analogue sample correspond well to that of a sebaceous-loaded fingermark, all the major peaks were present in relatively accurate quantities, the exception being the 1º amine peak at ~1600cm^{-1} in the analogue sample. This band represents the amino acid serine that was a representative for all the amino acids present in a natural fingermark. This band is not present in natural fingermarks, instead the 1º and 2º amide peaks from peptides and proteins dominate this region. Due to the stability and unreactive nature of amino acids within latent fingermarks, only degrading at >100ºC, and not undergoing photo-degradation [13, 15] it was felt that this would have a minimal effect on the dynamics of the analogue composition.

Once it was established that the IR spectra of the ‘complete’ analogue sample accurately represented that of the sebaceous-loaded fingermark further samples were created, each

with a component from the ‘complete’ analogue removed. As would be expected removing a component from the sample had a corresponding effect on the spectrum for that sample. For example, removing serine resulted in an absence of the corresponding absorption band at ~1600cm^{-1}. Equally, removing the wax ester myristyl myristate resulted in an observable reduction in the antisymmetric and symmetric CH_2 stretching modes at 2920 cm^{-1}and 2854 cm^{-1}, the C=O stretch mode at 1736 cm^{-1}, and the CH_2 bend at 1462 cm^{-1}, although the peak distribution within the spectra remained the same. Removing lactic acid from the sample had no observable effect on the spectra, this was to be expected given the small amount of the compound within the sample, also the functional groups within lactic acid, namely the hydroxide and carbonyl groups, are present in a many of the other components within the analogue sample as well as in natural fingermarks.

The 2920 cm^{-1} and 2854 cm^{-1} antisymmetric and symmetric C-H stretching modes of CH_2 groups are a combination of all the organic components within a sample. Removing key constituents such as wax esters or fatty acids had an impact on the height of these absorbance bands, and therefore quantity, as would be expected. As discussed earlier a cause of variation in band width are intermolecular interactions, which, in this study did not significantly vary from the ‘complete’ analogue sample in the absence of the wax ester, fatty acid, amino acid, mixed triglycerides or lactic acid, although peak height was affected, the coupling effect of the C-H stretch mode was not (figure 33). However, the absence of the steroid precursor squalene and the sterol cholesterol modified the coupling effect of the C-H stretching modes significantly (figure 34).

It could be reasoned that the absence of cholesterol and squalene would reduce peak height and therefore overall peak area simply due to a reduction in quantity of the sample analysed. Peak height however is not significantly different in the absence of these two components and therefore changes in peak area are directly proportional to peak width and therefore bond diversity (figure 34). Additionally, a reduction in peak height alone is not sufficient to explain this decrease in overall peak area in the absence of squalene or cholesterol. These components made up a small proportion of the overall sample, and as figure 34 shows, the absence of these components had no observable effect on peak height and therefore concentration. Also, it is only in the absence of squalene and cholesterol that such distinct C-H stretching modes, with minimal coupling, are generated.

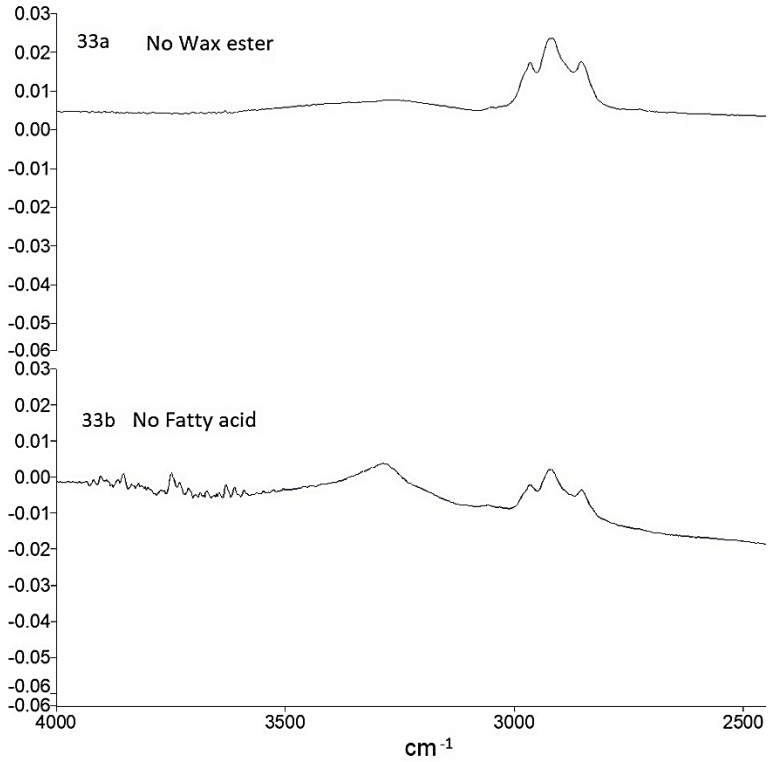

Figure 33: The antisymmetric and symmetric C-H stretch modes of CH_2 groups for analogue samples with the wax ester removed (33a), and with the fatty acid removed (33b).

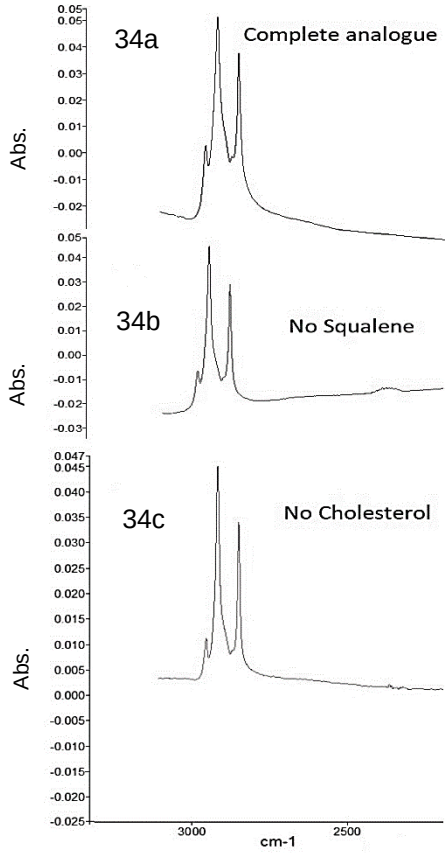

Figure 34: The antisymmetric and symmetric C-H stretch modes of CH_2 groups for a 'complete' analogue sample (34a), with squalene removed (34b), and with cholesterol removed (34c).

The antisymmetric and symmetric C-H stretching modes of CH_2 groups in the absences of squalene and cholesterol (figure 34b & 34c) show two distinct peaks with little overlap when compared to the coupling of the same peaks from the 'complete' analogue (figure 34a). This indicates that the intermolecular interactions in the absence of squalene and cholesterol are significantly reduced in these analogue samples.

Peak area calculations of the antisymmetric and symmetric C-H stretching modes of CH_2 groups quantify the spectral data and show that analogue samples, in the absence squalene or cholesterol, have a significantly lower absorbance between 2995 – 2780 cm^{-1} than that of the other analogue samples (figure 35).

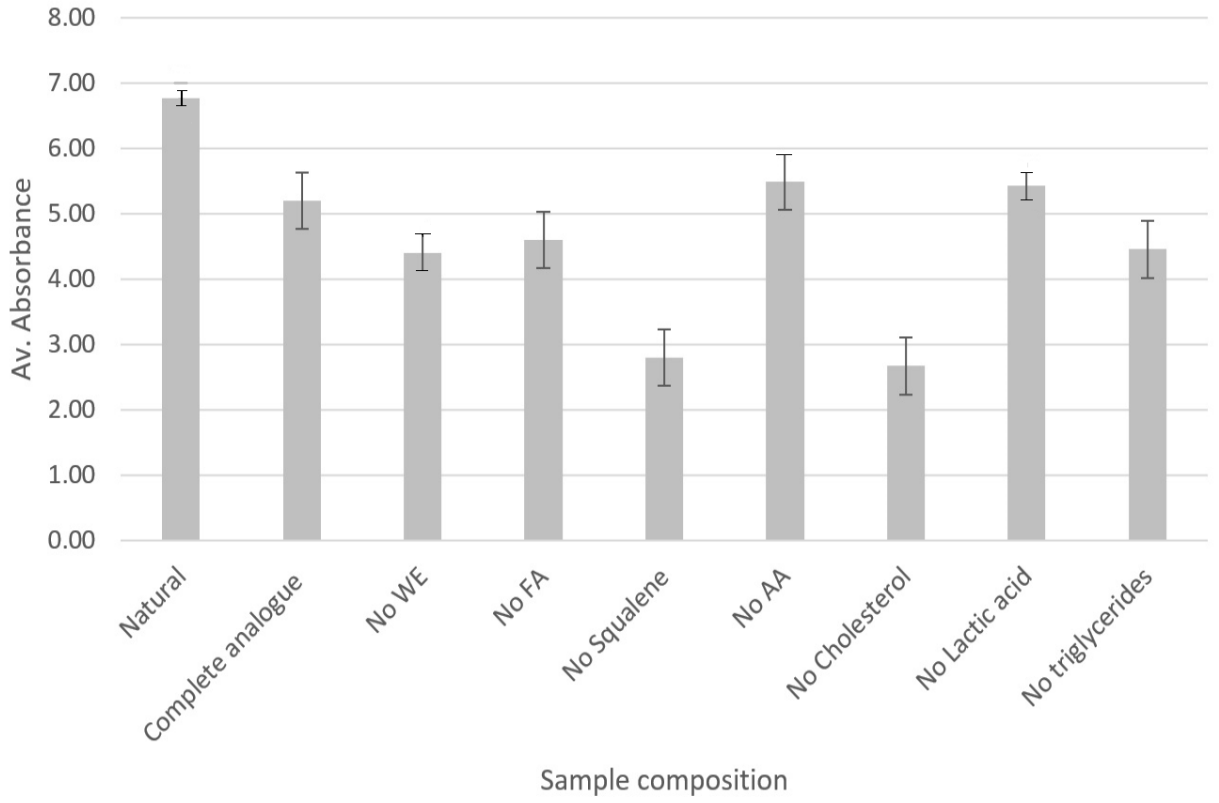

Figure 35: Average absorbance of the C-H stretching modes at 2995 - 2780 cm^{-1} of the analogue samples and natural fingermarks. (Natural fingermark n = 200, Analogues n= 20, error bars represent standard error of the mean)

The analogue samples investigated in this study suggest that the presence of both squalene and cholesterol are essential in driving intermolecular interactions within these samples. This could provide a focus for further research looking at the importance of these compounds within the dynamic environment of latent fingermark chemistry, and if squalene and cholesterol have an integral role in the aging processes within natural fingermarks.

As mentioned previously cholesterol can influence the degradation of triglycerides producing both saturated and unsaturated fatty acids [10]. The absence of this sterol could significantly slow the degradation of these other lipid compounds and reduce the quantity of degradation end products such as fatty acids. In a natural fingermark, saturated fatty acids appear to increase during the first few days after deposition [3]. The hydroperoxides and hydroxyl radicals produced during the oxidation of squalene

are also likely to affect the degradation of cholesterol and unsaturated fatty acids, and thus have a concomitant effect upon triglyceride decomposition and the initial increase in saturated fatty acids (figure 36).

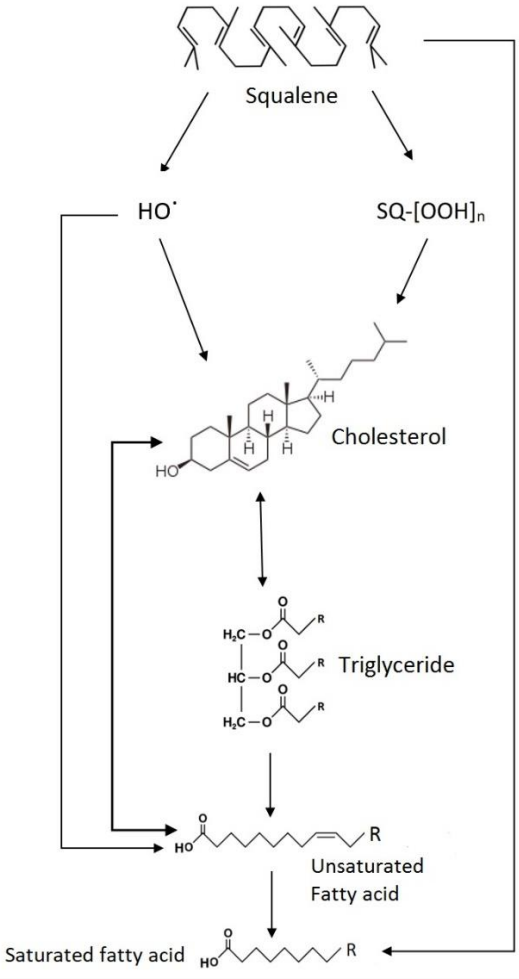

Figure 36: Suggested interaction pathway of lipid compounds within a latent fingermark.

Assuming that squalene and cholesterol are essential in driving the interactions between the various sebaceous compounds present in a latent fingermark, then this may, at least in part, explain why children's latent fingermarks are more volatile and tend to 'vanish' instead of adopting the temporal degradation of adult fingermarks [44–46]. Children's latent fingermarks contain very little squalene so interactions between this steroid precursor and other sebaceous compounds (such as unbranched fatty acids) would be limited. Reduced interactions would mean that degradation of these other sebaceous compounds (particularly the more volatile ones) to more stable states

would be reduced leaving them more prone to evaporation. The sebaceous components of young children also contain significantly lower levels of triglycerides than that of adults, so the breakdown of cholesterol and fatty acids through interactions with triglycerides (and vice versa) to more stable end products would also be inhibited.

6.5 Conclusions

The research presented in this paper demonstrates that squalene and cholesterol affect the intermolecular interactions within the analogue samples studied, this could indicate the importance of these two compounds in influencing intermolecular interactions between lipids within latent fingermarks, and provides a hypothesis for studying these interactions within natural fingermarks. The degradation processes that occur in latent fingermarks are directed by these interactions and only by studying them will it be possible to understand the complex and dynamic nature of fingermark aging. Future studies using analogue compositions could investigate whether these analogue samples age in similar ways to natural fingermarks by identifying known degradation products that occur in natural latent fingermarks. Analogue compositions of latent fingermarks will always be crude representations of the myriad of interactions that occur within natural fingermarks, but ongoing investigation into these simplified models, utilising various analytical techniques, could direct research that ultimately provides a better understanding of the fingermark aging process.

6.6 References

1. K. A. Mountfort, H. Bronstein, N. Archer, S. M. Jickells, Identification of oxidation products of squalene in solution and in latent fingermarks by ESI-MS and LC/APCI-MS, *Anal. Chem.* 79 (7) (2007) 2650–2657.

2. G. M. Mong, C. E. Petersen, and T. R. W. Clauss, Advanced Fingerprint Analysis Project - Fingerprint Constituents, Vol PNNL-13019. U.S. Department of Energy, Pacific Northwest National Laboratory, 1999.

3. N. E. Archer, Y. Charles, J. a. Elliott, and S. Jickells, Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface, *Forensic Sci. Int.* 154 (2-3) (2005) 224–239.

4. R. S. Croxton, M. G. Baron, D. Butler, T. Kent, and V. G. Sears, Variation in amino acid and lipid composition of latent fingerprints, *Forensic Sci. Int.* 199 (2010) 93–102.

5. R. Ramotowski, *Composition of Latent Print Residues, in: Advanced in Fingerprint Technology, 2nd ed.* London: CRC Press, 2001.

6. A. Srivastava and R. Prasad, Triglycerides-based diesel fuels, *Renew. Sust. Energy. Rev*, 4 (2) (2000) 111–113.

7. B. B. Dent, S. L. Forbes, and B. H. Stuart, Review of human decomposition processes in soil, *Environmental Geology*. (2004) 576-585.

8. R. Wolstenholme, R. Bradshaw, M. R. Clench, and S. Francese, Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids, *Rapid Commun. Mass Spectrom.* 23 (19) (2009) 3031–3039.

9. C. Weyermann, C. Roux, and C. Champod, Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis, *J. Forensic Sci.* 56 (1) (2011) 102–108.

10. L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, *Chemistry and Phys of Lipids*, 164 (6) (2011) 457-468.

11. S. K. Kim and W. W. Nawar, Oxidative interactions of cholesterol with triacylglycerols, *J. Am. Oil Chem. Soc*, 68 (12) (1991) 931–934.

12. A. Girod, R. Ramotowski, and C. Weyermann, Composition of fingermark residue: A qualitative and quantitative review, *Forensic Sci. Int.*, 223 (1–3) (2012) 10–24.

.13. T. Atherton, R. Croxton, M. Baron, J. Gonzalez-Rodriguez, L. Gámiz-Gracia, and A. M. García-Campańa, Analysis of amino acids in latent fingerprint residue by capillary electrophoresis-mass spectrometry, *J. Sep. Sci.* 35 (2012) 2994-2999.

14. A. Stadtman, A. Cherkes, C. Anfinsen, Studies on the microbiological degradation of cholesterol, *J. Biol. Chem.* 206 (2) (1954) 511–523.

15. A. Richmond-Aylor, S. Bell, P. Callery, and K. Morris, Thermal degradation analysis of amino acids in fingerprint residue by pyrolysis GC-MS to develop new latent fingerprint developing reagents, *J. Forensic Sci.* 52 (2) (2007) 380–382.

16. G. De Paoli, S. A. Lewis, E. L. Schuette, L. A. Lewis, R. M. Connatser,T. Farkas, Photo- and thermal-degradation studies of select eccrine fingerprint constituents, *J. Forensic Sci.* 55 (4) (2010) 962–969.

17. S. K. Kim and W. W. Nawar, Parameters influencing cholesterol oxidation, *Lipids*. 28 (10) (1993) 917–922.

18. L. L. Smith, Cholesterol autoxidation 1981–1986, *Chem. Phys. Lipids*. 44 (2–4) (1987) 87–125.

19. A. Girod, L. Xiao, B. Reedy, C. Roux, and C. Weyermann, Fingermark initial composition and aging using Fourier transform infrared microscopy (μ-FTIR), *Forensic Sci. Int.* 254 (2015) 185-196.

20. P. Fritz, W. van Bronswjik, K. Lepkova, S. W. Lewis, K. F. Lim, D. E. Martin, L. Puskar, K. F. Lim, D. E. Martin, L. Puskar, K. F. Lim, D. E. Martin, and L. Puskar, Infrared microscopy studies of the chemical composition of latent fingermark residues, *Microchem. J.* 111 (2013) 40–46.

21. C. Ricci and S. G. Kazarian, Collection and detection of latent fingermarks contaminated with cosmetics on nonporous and porous surfaces, *Surf. Interface Anal.* 42 (5) (2010) 386–392.

22. D. K. Williams, R. L. Schwartz, and E. G. Bartick, Analysis of Latent Fingerprints Deposits by Infrared Microspectroscopy, *Appl. Spectrosc.* 58 (3) (2004) 313–316.

23. N. J. Crane 3, E. G. Bartick, R. S. Perlman, and S. Huffman, Infrared spectroscopic imaging for noninvasive detection of latent fingerprints, *J. Forensic Sci.* 52 (1) (2007) 48–53.

24. A. Hemmila, J. McGill, and D. Ritter, Fourier transform infrared reflectance spectra of latent fingerprints: a biometric gauge for the age of an individual, *J. Forensic Sci.* 53 (2) (2008) 369–376.

25. A. Johnston and K. Rogers, The Effect of Moderate Temperatures on Latent Fingerprint Chemistry, *Appl. Spectrosc.* 71 (9) (2017) 2102-2110.

26. N. B. Colthup, L. H. Daly, S. E. Wiberley, N. B. Colthup, L. H. Daly, and S. E. Wiberley, “Chapter 4 – The Vibrational origin of group frequencies, in *Introduction to Infrared and Raman Spectroscopy*. (1990) 171–213.

27. G. Socrates, Infrared Characteristsic Group Frequencies. Tables and Charts, Second Ed. John Wiley & Son Ltd. (1994) 34–41.

28. F. M. Fowkes, Donor-Acceptor Interactions at Interfaces, *J. Adhes.* 4 (2) (1972) 155–159.

29. N. Israelachvili, J. N., Tabor, The Measurement of Van Der Waals Dispersion Forces in the Range 1.5 to 130 nm, in *Proceedings of The Royal Society A*, (1972) 19-38.

30. J. Israelachvili, *Intermolecular and Surface Forces*, 3rd ed. Elsevier, 2011.

31. X. Li, Q. Pan, J. Chen, S. Liu, A. He, C. Liu, Y. Wei, K. Huang, L. Yang, J. Feng, Y. Zhao, Y. Xu, Y. Ozaki, I. Noda, and J. Wu, Asynchronous Orthogonal Sample Design Scheme for Two-Dimensional Correlation Spectroscopy (2D-COS) and its Application in Probing Intermolecular Interactions from Overlapping Infrared (IR) Bands, *Appl. Spectrosc.* 65 (8) (2011) 901–917.

32. J. Chen, Q. Bi, S. Liu, X. Li, Y. Liu, Y. Zhai, Y. Zhao, L. Yang, Y. Xu, I. Noda, and J. Wu, Double Asynchronous Orthogonal Sample Design Scheme for Probing Intermolecular Interactions, *J. Phys. Chem.* 116 (45) (2012) 10904–10916.

33. N. B. Colthup, L. H. Daly, S. E. Wiberley, N. B. Colthup, L. H. Daly, and S. E. Wiberley, Chapter 5 – Methyl and Methylene groups, in *Introduction to Infrared and Raman Spectroscopy*. (1990) 215–233.

34. A. L. Steber, J. L. Neill, D. P. Zaleski, B. H. Pate, A. Lesarri, R. G. Bird, V. Vaquero-Vara, and D. W. Pratt, Structural studies of biomolecules in the gas phase by chirped-pulse Fourier transform microwave spectroscopy, *Faraday Discuss.* (2011) 227-242.

35. B. K. Sharma, *Instrumental Methods of Chemical Analysis*, Twenty Fourth Edition. Meerut: GOEL, 2005.

36. J. Almog, A.A. Cantu, C. Champod, T. Kent, C. Lennard, Guidelines for the assessment of fingermark detection techniques. International Fingerprint Research Group (IFRG), *J. Forensic Ident.*, 64, (2) (2014) 174–200

37. A. Girod, C. Weyermann, Lipid composition of fingermark residue and donor classification using GC/MS, *Forensic Sci. Int.*, 238 (2014) 68–82

38. S. Zadnik, W. Van Bronswijk, A. Frick, P. Fritz, S.W. Lewis, Fingermark simulants and their inherent problems: A comparison with latent fingermark deposits, *J. Forensic. Ident.*, 63 (5) (2013) 593–608.

39. M. J. Bailey , N. J. Bright, S. Hinder, B. N. Jones, R. P. Webb, R. S. Croxton, S. Francese, L. S. Ferguson, R. Wolstenholme, S. Jickells, B. J. Jones, J. J. Ojeda, S. G. Kazarian, and S. Bleay, Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spec, *Anal. Chem.* 84 (20) (2012) 8514–8523.

40. S. Cadd, M. Islam, P. Manson, and S. Bleay, Fingerprint composition and aging: A literature review, *Sci. Justice*. 55 (4) (2015) 219–238.

41. P. Hamilton, Amino-acids on Hands, *Nature*. 205 (1965) 284–285.

42. J. Oro and H. Skewes, Free Amino-Acids on Human Fingers: The Question of Contamination in Microanalysis, *Nature*. 207 (1) (1965) 1042–1045.

43. B. Hadorn, F. Hanimann, P. Anders, H. Curtius, and R. Halverson, Free Amino-acids in Human Sweat from Different Parts of the Body, *Nature*. 215 (99) (1965) 416–417.

44. K. M. Antoine, S. Mortazavi, L. M. Miller, A. D. Miller, K. M. Antoine, S. Mortazavi, A. D. Miller, L. M. Miller, Chemical differences are observed in children's versus adults' latent fingerprints as a function of time, *J. Forensic Sci.* 55, (2) (2010) 513–518.

45. D. K. Williams, C. J. Brown, and J. Bruker, Characterization of children's latent fingerprint residues by infrared microspectroscopy: Forensic implications, *Forensic Sci. Int.* 206 (1–3) (2011) 161–165.

46. D. Noble, Vanished into thin air. The search for children's fingerprints, *Anal. Chem.* 67 (13) (1995) 435A–438A.

CHAPTER 7. PAPER FOUR

(Submitted to Forensic Science International – under review)

The temporal degradation of illicit contaminants in latent fingermarks using Fourier transform infrared spectroscopic imaging

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7.1 Abstract

Fourier Transform Infrared (FTIR) spectroscopy has been shown to be a rapid, non-destructive analytical technique capable of detecting trace amounts of exogenous particulate in fresh latent fingermarks. Fingermarks identified at a crime scene, however, are rarely fresh and are likely to have been deposited days or even weeks before forensic analysis, leaving them prone to decomposition.

In this study latent fingermarks were contaminated with trace amounts of the improvised explosive precursors ammonium nitrate and sodium chlorate, and the addictive narcotic cocaine. The latent fingermarks were then aged in natural daylight conditions over a 30-day period and intermittently analysed using FTIR spectromicroscopy. Exogenous particulate was identified using spectroscopic imaging of each fingermark, and spectra obtained from the samples were compared to control spectra to confirm the identification of the contaminant. This study demonstrates that these contaminants are detectable within latent fingermarks up to at least 30 days after deposition, with only ammonium nitrate showing signs of decomposition, and that fingermark chemistry has a minimal effect on their molecular integrity. This information has broad implications for the law enforcement community as it suggests that the detection of exogenous particulate within latent fingermarks is possible long after a suspect has handled an illegal substance. This study also confirms the use of spectroscopic imaging to provide a chemical signature for these illicit compounds weeks after deposition, and an image of the fingermark ridge pattern that can then be used for identification purposes.

Keywords

Fingermarks, Fourier transform infrared microspectroscopy, spectroscopic imaging, degradation, exogenous material, detection, explosives, narcotics

7.2 Introduction

The ability to detect illicit substances such as explosive compounds/precursors or narcotics in latent fingermarks is of obvious benefit to the forensic and law enforcement communities. This is of particular relevance to today's threat lists with the increased use of improvised explosive devises (IED's) by terrorist groups, often formulated from domestically available products, and the rising trend in cocaine consumption in western Europe and the US.

Various analytical techniques have proven capable of detecting trace amounts of illicit materials, particularly in latent fingermarks, such as gas chromatography-mass spectroscopy (GC-MS), Matrix-assisted laser desorption/ionisation-mass spectrometry (MALDI-MS), high performance liquid chromatography (HPLC), capillary electrophoresis, and ion mobility spectroscopy (IMS) [1-7]. These techniques, however, do require the destruction of the sample for analysis, leaving further investigation of the fingermark impossible. Whereas spectroscopic techniques such as Raman and Infrared spectroscopy are capable of detecting these illicit materials rapidly, with limited or no sample preparation, and, most importantly, are non-destructive with regards to the sample [8-17]. IR spectromicroscopy in particular has demonstrated the ability to identify exogenous particulate within latent fingermarks by generating identifiable spectra [9, 10,15], but is also capable of generating spectral images, or maps, that provide spatial information about a fingermark [18-24]. These spatial images can both identify the chemical signatures of foreign components within a fingermark, and also provide the spatial information of the unique ridge patterns that could be used to identify a suspect.

The dynamic nature of fingermark chemistry post-deposition is a complex process, numerous studies have examined the chemical composition and aging of fingermarks, the oxidation mechanisms that take place, both short and long term [25, 26], and the resulting end products [26-36]. For example, it is well documented that the chemistry of latent fingermarks changes temporally in both adults and children [37-40] and this may potentially provide the basis for an aging tool to establish time since deposition.

The chemical components of latent fingermarks have been well documented using a variety of analytical techniques [41, 42]. Previous studies have shown that there are

changes in fingermark chemistry over time and under different conditions. Temporal decomposition, for example, appears to involve the shortening and degradation of unsaturated lipids including fatty acids, wax esters, triglycerides, squalene (SQ) and cholesterol due to various oxidation processes [25-28, 30-36, 43-45]. In contrast, saturated lipids stay relatively stable over longer time periods (>60 days) [26]. A change in unsaturated fatty acids has been observed over time decreasing significantly over a thirty-day period [26, 27]. This is due to the unsaturated moiety degrading through both aerobic and anaerobic processes and increasing the proportion of saturated compounds [26]. Exposure to light also has a significant impact on fingermark composition. Studies have shown that exposure to light affects the breakdown mechanisms of various components within fingermark deposits differently and more rapidly than in dark conditions. Short chain fatty acids and squalene in particular were affected by exposure to light conditions [27, 43, 46]. SQ degradation is supported by the identification of various photo-oxidation mechanisms to produce intermediary products including peroxides, hydroperoxides and SQ epoxide and the fully oxidised forms being hexanedioic and pentanedioic acid [25]. These products are particularly prevalent when squalene is exposed to UV radiation, such as direct sunlight [25, 46].

The identification and chemical imaging of exogenous components within latent fingermarks, as well as the composition and aging of latent fingermarks has been, and continues to be, comprehensively studied. Investigations into the temporal degradation of foreign substances within fingermarks has largely been overlooked however. This is an important aspect of latent fingermark analysis as fingermarks found at crime scenes are rarely fresh, and could have been deposited days or weeks prior to investigation by law enforcement agencies. It is vital, therefore, to understand the effects of fingermark degradation chemistry on trace evidence over time. This study aims to address this gap in the literature by using FTIR spectromicroscopy to analyse the temporal degradation of three exogenous substances within latent fingermarks over a 30-day period. FTIR spectromicroscopy is ideally suited to monitoring temporal changes in latent fingermarks as specific sample areas can be repeatedly analysed over the duration of the experiment [17, 40, 47].

Three substances were selected to contaminate the fingermarks; ammonium nitrate (AN) and sodium chlorate (SC), both common pre-cursor components in IED's, and the addictive stimulant narcotic cocaine (C). Fingermarks were contaminated with each one of these compounds and aged in natural light conditions over 30 days. The same sample area was analysed and a chemical map generated every 5 days for the duration of the experiment. Spectra of individual particles from each contaminant were also obtained at five day intervals to observe any changes in specific absorbance bands as the exogenous compounds interacted with the chemical makeup of the fingermarks.

7.3 Experimental

To prepare the fingermark samples the hands of the volunteer were cleaned with soapy water, rinsed and air dried for twenty minutes. The index finger of the right hand was drawn from the bridge of the nose to the temple five times to collect sufficient sebaceous secretions to simulate natural grooming behaviour. The volunteer's finger was then pressed into 500 mg of the contaminant, any excess was brushed off with the other hand (gloved) until little or no powder was visible on the fingertip. The contaminated finger was then lightly pressed onto a CaF_2 Infrared (IR) microscope slide (10 mm x 10 mm, Crystran Ltd). Five fingermarks per contaminant were laid down on consecutive days and deposited between 9am and 10am to avoid significant diurnal variations in composition of the latent fingermarks. For each fingermark the same index finger was used. An uncontaminated control fingermark was deposited on an identical CaF_2 microscope slide, and five deposits were analysed from that sample. Control samples of 10 mg of each contaminant were deposited on three separate CaF_2 slides without the fingermark, five deposits were analysed from each slide. All sample fingermarks were obtained from a single male donor, 38 years old, to maintain a relative consistency between samples.

Contaminants: Ammonium nitrate ≥99% (Sigma Aldrich, UK), sodium chlorate ≥98% (Sigma Aldrich, UK), and cocaine free base C-II ≥99% (Sigma Aldrich, UK), milled to a fine powder.

7.3.1 Analysis of fingermarks

The fingermarks were analysed using a PerkinElmer Spectrum™ Spotlight 200 FTIR imaging System equipped with a liquid-nitrogen cooled MCT linear array detector. Data was analysed using Perkin Elmer software, Spectrum® (v10.02.00), variations in spectra were processed using peak area calculations. All spectra were baseline corrected and range normalised. Spectra from control samples and contaminated fingermarks were collected in transmission mode, over the 4000 to 750 cm^{-1} spectral range at 4 cm^{-1} spectral resolution and 10 µm spatial resolution, after 32 scans, using a 100 x 100 µm aperture. All spectra were presented in units of absorbance ($A = -\log T$) as a function of wavenumber, the number of waves per centimetre. As with the control samples five deposits were selected for spectral analysis from each contaminated fingermark. Each

fingermark and control sample was analysed every 5 days with both a visible image scan (5000x5000 µm), consisting of 169 (13x13) tiles, and a corresponding chemical map obtained from each sample. Before analysis began, contaminant particles were selected using the visual microscope capability and that area was sampled for the duration of the 30-day experiment. The same location was identified for each analysis by returning to the same area on the slide and identifying a characteristic group of particulate. The chemical maps allowed for individual spectra from each contaminant particle to be obtained and any changes in absorbance observed over the duration of the experiment. Changes in absorbance for the control samples and the contaminants within the fingermarks were calculated by selecting the three most dominant absorbance bands from each compound (table 8), measuring their peak area after each scan, and averaging each sample. All samples were stored in natural light conditions at room temperature and humidity. All statistical tests were carried out using Minitab 18 software.

Sample	Absorbance bands selected for analysis (cm^{-1})			
		Band 1	**Band 2**	**Band 3**
	Ammonium nitrate	1628	1044	828
	Sodium chlorate	1887	1595	1415
	Cocaine	2946	1735	1449
	Fingermark	2925	2858	1738

Table 8: The three absorbance bands selected for analysis from the contaminants and the latent fingermarks over the duration of the experiment

7.4 Results and Discussion

7.4.1 Obtaining spectra and chemical maps

Before the contaminated fingermarks were analysed control spectra were obtained from pure samples of the contaminants on day 0 and day 30 (figure 37). Five deposits on each of the three slides containing 10 mg of each contaminant were analysed. This was to identify any changes in absorbance, indicating degradation, that were unrelated to interactions with fingermark chemistry. The spectral range of analysis for all samples was 4000 to 750 cm^{-1} with the exception of ammonium nitrate. The 'fingerprint region' of the ammonium nitrate spectrum (2500 cm^{-1} - 750 cm^{-1}) was selected for analysis for both the control and contaminated fingermark samples as this region provided the most accurate data on changes in absorbance over time. As figure 37 shows there was no significant change ($p > 0.05$) in the absorbance of the three control contaminants.

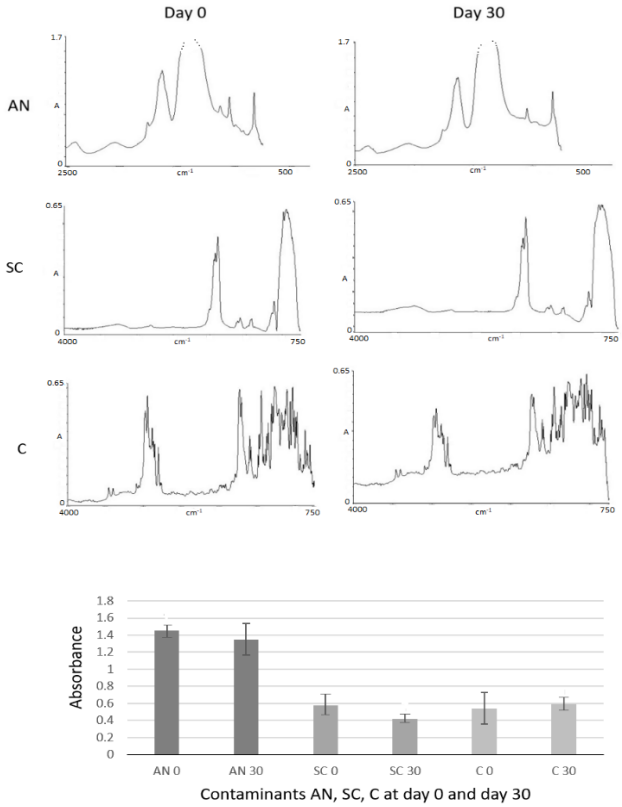


Figure 37: Averaged control spectra from five deposits of each of the contaminants; ammonium nitrate (AN), sodium chlorate (SC), and cocaine (C) on day 0 and day 30 (n=15 error bars represent standard error of the mean).

Control spectra and chemical maps of the uncontaminated latent fingermark were also obtained to measure the chararcteristic peaks, and any observable changes that occured over the 30-day period (figure 38).

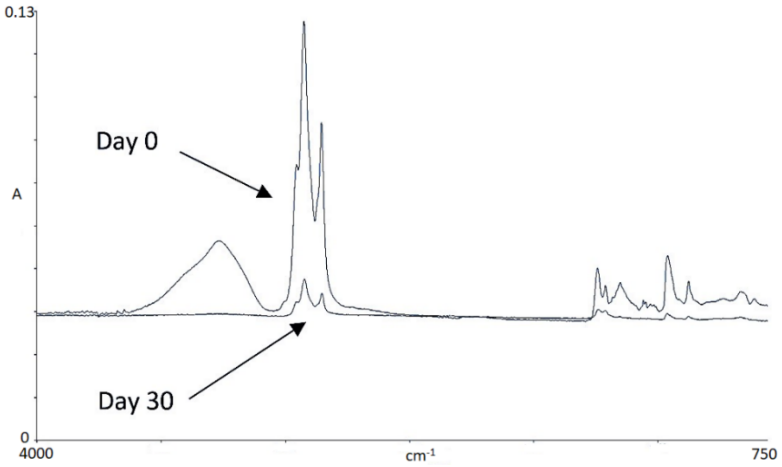


Figure 38: Averaged spectra of an uncontaminated latent fingermark at day 0 and day 30 (n=5)

There were no significant differences (p = 0.13) in the selected absorbance bands from the control sample contaminants on day 0 and day 30 (figure 37). This indicated that any changes in absorbance from the exogenous particulate within the fingermarks would be due to interactions with the fingermark chemistry.

The IR spectra in figure 38 show a general reduction in peak area, and therefore quantity, across all bands of the uncontaminated control fingermark over the 30-day period, and notably the OH band at 3260 cm^{-1} showed a significant reduction, most likely due to water loss. Figure 39 shows that the absorbance of the antisymmetric and symmetric C-H stretching modes of CH_2 groups (2920-2854 cm^{-1}) significantly decreased over the 30 days, as would be expected.

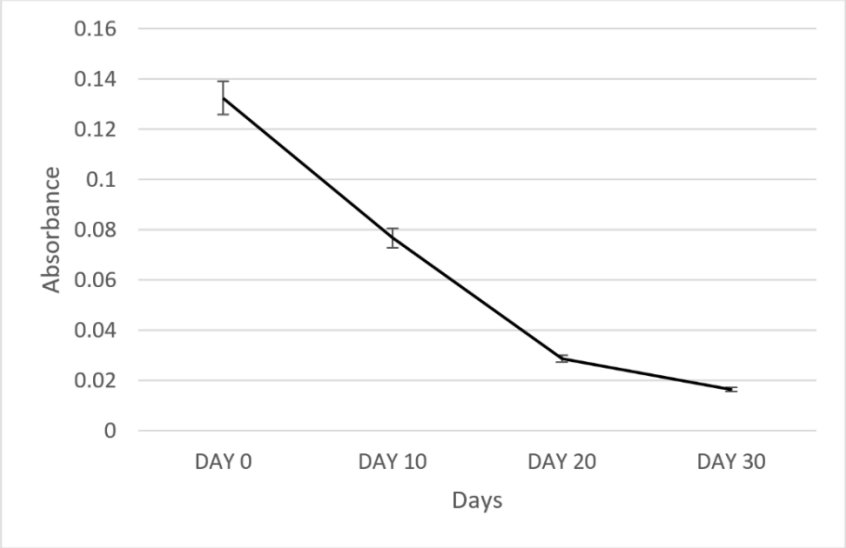


Figure 39: Change in absorbance of the C-H stretching modes of CH_2 groups of the control fingermark (uncontaminated) over 30 days (n=5, error bars represent standard error of the mean)

This band is a culmination of all the organic material within a latent fingermark, and as mentioned previously various oxidation mechanisms take place within a latent fingermark over time and materials such as triglycerides, wax esters, short chain fatty acids, and squalene decrease in quantity, especially in light conditions [25, 26, 27].

Figure 40 shows the visible image and corresponding chemical map (chemimap) of the same area of a latent fingermark contaminated with AN. The AN particles observed in the visible image show strong absorbance in the chemimap.

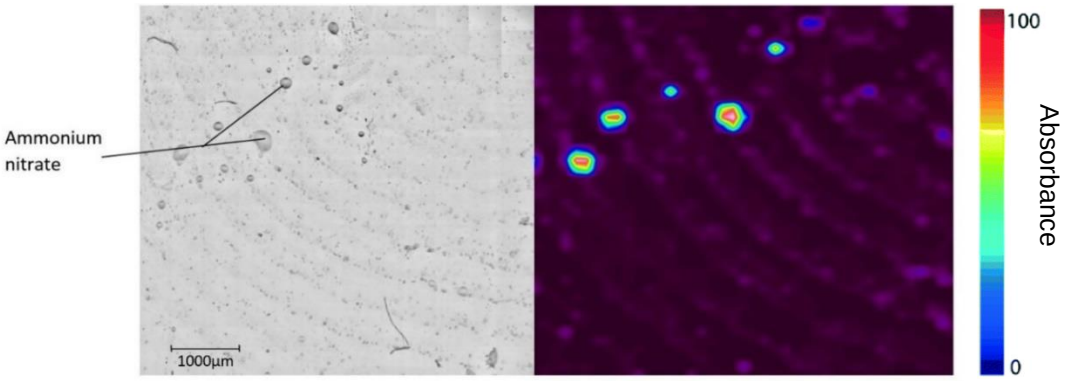


Figure 40: Visible image and corresponding chemimap of a latent fingermark contaminated with ammonium nitrate particles

Figure 41 a-c shows the indiviual spectra obtained from each of these AN particles, as well as a background spectrum of the endogenous fingermark (41d). The spectra confirm the identity these particles as ammonium nitrate when compared to the control spectrum for AN (41e). This process was repeated for the fingermarks contaminated with sodium chlorate and cocaine.

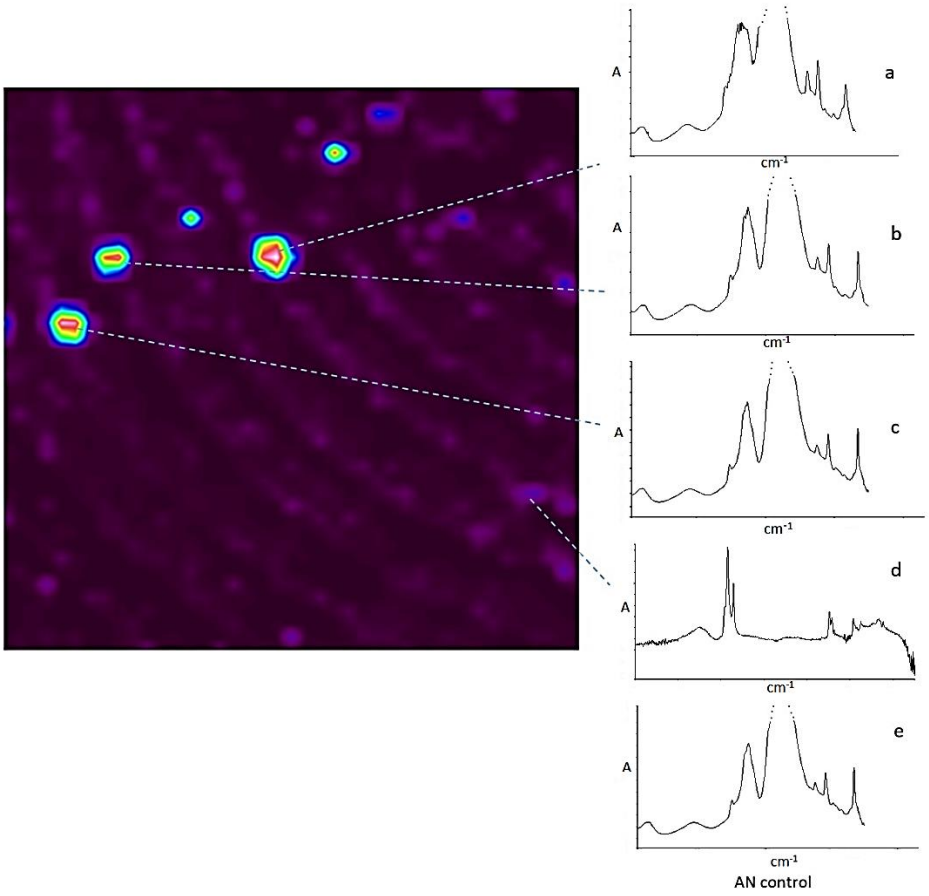


Figure 41: Chemimap and spectra of AN in a latent fingermark. 41a, b and c show consistent spectra of ammonium nitrate particles present within the fingermark when compared to the control spectrum of AN (41e). 41d shows a typical spectrum of a fingermark from the donor used in this study.

7.4.2 Aged sample analysis using spectroscopic imaging

The objective of this study was to investigate the degradation effects of fingermark chemistry on different contaminants over a 30-day period using FTIR spectroscopic imaging. The previous findings (7.4.1) demonstrate that there is no significant degradation of the contaminant control samples over time (figure 37), and that it is possible to reliably detect exogenous particulate within latent fingermarks using chemical mapping (figures 40 & 41).

Figure 42 shows two examples of spectroscopic images of latent fingermarks contaminated with ammonium nitrate (42A) and cocaine (42B) on day 0 and day 30.

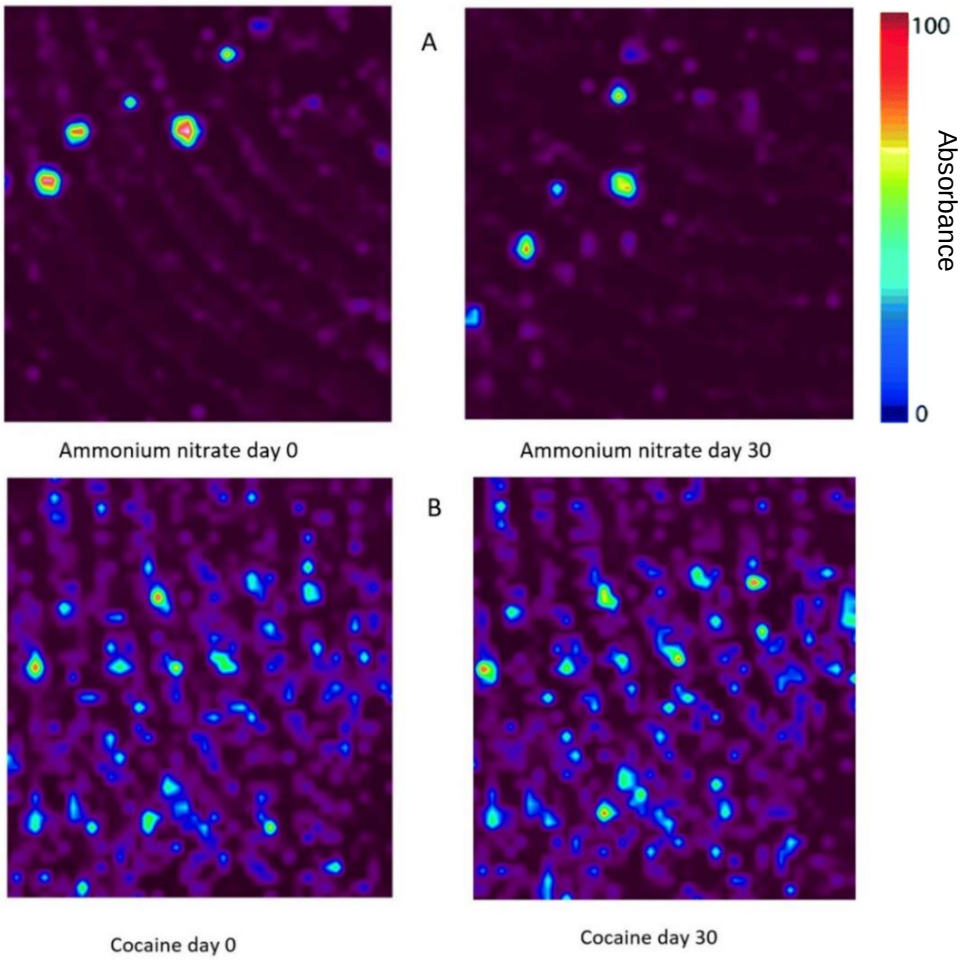


Figure 42: Chemimap comparison of the degradation of ammonium nitrate (A) and cocaine (B) on day 0 and day 30

In Figure 42A the chemimaps show an observable difference in the average absorbance, and therefore quantity of material, of ammonium nitrate from day 0 to day 30, but there is no obvious difference between the chemimaps of fingermarks contaminated with cocaine (42B). This reduction in average absorbance between day 0 and day 30 for ammonium nitrate, as shown in figure 42A, was confirmed by the peak area calculations from the spectral data (figure 43), but these changes were not seen in the averaged spectra of the sodium chlorate or cocaine samples. N.B. The saturated nitrate ion band at 1410-1340 cm^{-1} is a natural consequence of absorption in the larger AN particulates, typical of that within fingermark contamination. This band was not used in any calculations for this study.

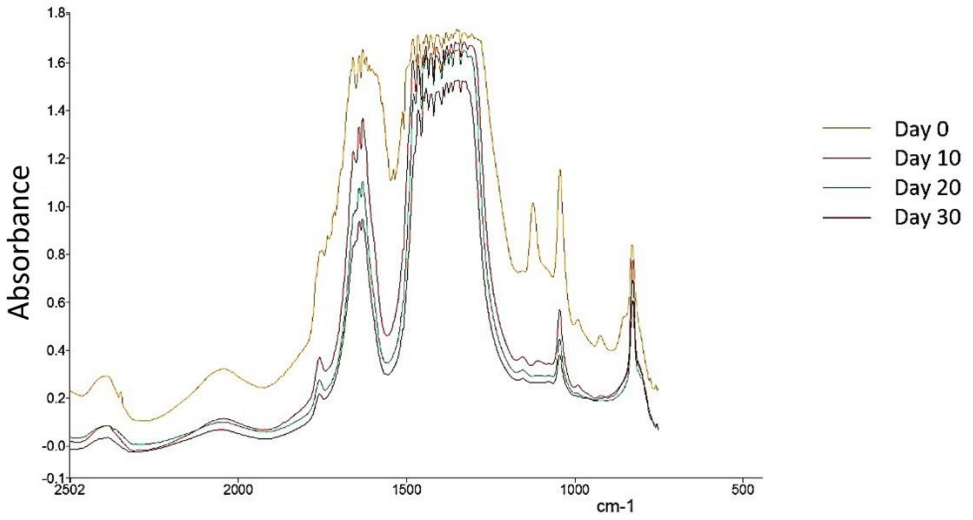


Figure 43: Changes in absorbance of AN over 30 days, focusing on absorbance bands at 1628, 1044, 828 cm^{-1} (n=25)

To establish any quantifiable changes in absorbance of the three contaminants over the 30-day period spectral data was analysed using peak area analysis, and ’s Rho Correlation (Minitab 18) was used to identify any significant correlation between time and average absorbance of the three contaminants. No significant correlation was observed for sodium chlorate (p = 0.2) or cocaine (p = 0.05), but there was a significant reduction in absorbance for ammonium nitrate (p < 0.01) over the 30-day period. Figure 44 plots the average absorbance for each of the three contaminants over the 30-day period. As confirmed by the Spearman’s Rho correlation only AN showed a significant decrease in

quantity, although this reduction occurred almost entirely within the first 10 days, and AN particulates were still detectable after 30 days (figure 42A & 43).

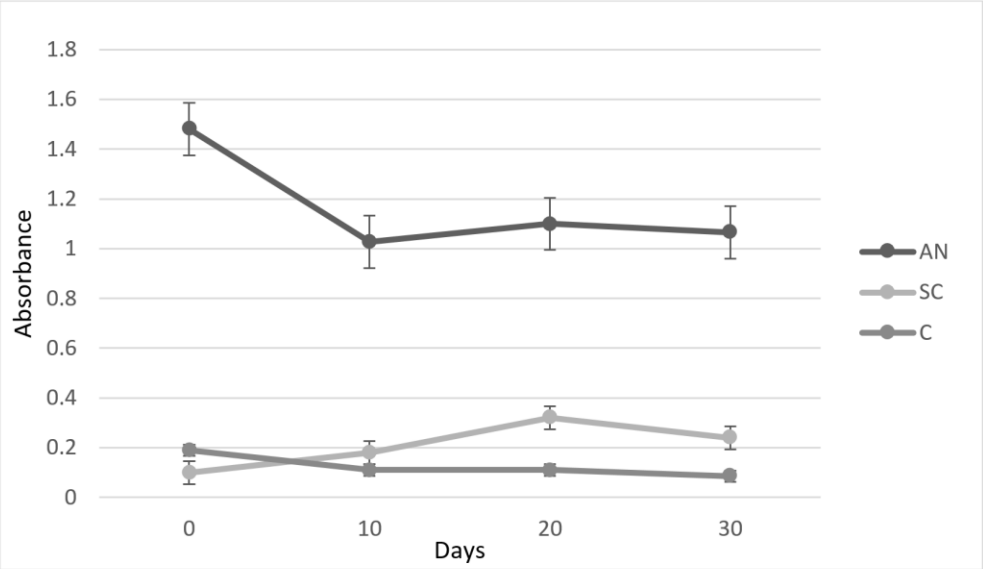


Figure 44: Changes in average absorbance over a 30-day period of ammonium nitrate (AN), sodium chlorate (SC) and cocaine (C) (n=25, error bars represent standard error of the mean)

Ammonium nitrate showed a 31% decrease in absorbance within the first ten days and then only a 4% decrease over the following twenty days. It is likely that this initial reduction in absorbance of AN is due to ionic interactions with both water molecules and endogenous unsaturated lipid components within the fingermark. It is known that these unsaturated lipids, such as squalene and unsaturated fatty acids, decompose within the first few days' post-deposition [26, 27, 36, 43]. Polar water molecules will break the ionic bonds within ammonium nitrate to form ammonium and nitrate ions, these ions would then be available to interact with the unsaturated lipids. This interaction between the inorganic ions and unsaturated lipids would also be assisted by the photo-oxidation mechanisms that are known to take place in light conditions [25, 27, 43, 46]. The lack of change in absorbance after the first ten days is likely to be in part due to the evaporation of water from the fingermark, and due to the near complete decomposition of these unsaturated lipids.

Ammonium nitrate and sodium chlorate are ionic compounds so it might be expected that they would both show some degradation over time in the aqueous and dynamic environment of a latent fingermark. Yet it was only AN that degraded over the 30-day period, SC remaining unchanged. This is likely due to the difference in lattice enthalpy of the two compounds. AN has a significantly lower lattice enthalpy than SC, so is more likely to form ions in solution in the ambient conditions of a latent fingermark. Cocaine is a covalently bonded alkaloid ester, so the lack of change in absorbance over time in this study is consistent with its intramolecular stability.

7.5 Conclusion

This study demonstrates that trace amounts of ammonium nitrate, sodium chlorate, and cocaine, are still detectable in latent fingermarks up to at least 30 days post-deposition in ambient conditions. It also reiterates the advantages of using FTIR spectromicroscopy to identify trace amounts of contaminant within latent fingermarks, and the ability to analyse any chemical changes in that contaminant over time without destroying the sample. All three contaminants were detectable after thirty days, only ammonium nitrate showed any significant reduction in quantity over the test period, primarily in the first 10 days**.** This study used one donor for the fingermarks, which, given the reported variability between individuals, could be considered limiting with regard to chemical variation. It was felt however, that for this study, this consistency was an advantage in assessing changes in contaminant composition over time. Further research should look into the effect of various donors on the chemical integrity of these contaminants, which may in addition provide more information on the chemical variability between individuals.

The variety of illicit contaminants, from either explosive/explosive precursors or narcotics, that could potentially be found in latent fingermarks is immensely broad. Although only three contaminants were selected for this study the data does suggest that any contaminant degradation that does occur is likely to arise within ten-days of fingermark deposition. This is when the naturally occurring oxidation mechanisms within a latent fingermark are most active and therefore intermolecular interactions with other species are more likely. It is also when the water content of a fingermark is at its highest enabling the more polar compounds to form a solution. It is worth noting that the fingermarks used for this study were sebaceous-loaded to represent natural grooming behavior. This loading however, can artificially enrich fingermarks with lipid components, and may affect degradation rates of certain contaminants given there are more lipid materials to interact with. Although even with sebaceous-loaded fingermarks the contaminants in this study were still detectable for the duration of the experiment.

In this work FTIR spectromicroscopy has demonstrated the ability to not only chemically identify trace amounts of illicit compounds within latent fingermarks weeks after

deposition, but also to simultaneously provide a recognizable ridge pattern that can be used for identification purposes without destroying the sample. This data has broad implications for the law enforcement community as the ability to detect illicit compounds in latent fingermarks a considerable time after deposition is potentially of great value to a criminal investigation, for example when tracing the movements of a terrorist or terrorist group synthesizing improvised explosive compounds. Further work is needed on a broader range of illicit substances to identify any compounds that are particularly susceptible to degradation within the chemical environment of a latent fingermark, and how varying environmental conditions could affect that rate of decomposition.

7.6 Reference

1. D. S. Moore, Instrumentation for trace detection of high explosives*. Rev. Sci. Instrum*. 75 (2004) 2499-2512.

2. M. Nambayah, T.I. Quickenden, A quantitative assessment of chemical techniques for detecting traces of explosives at counter-terrorist portals. *Talanta*. 63 (2004) 461-467.

3. S. Singh, Sensors—An effective approach for the detection of explosives. *J. Hazard Mater*. 144 (2007) 15-28.

4. H. Lai, A. Leung, M. Magee, J.R. Almirall, Identification of volatile chemical signatures from plastic explosives by SPME-GC/MS and detection by ion mobility spectrometry. *Anal. Bioanal. Chem*. 396 (2010) 2997-3007.

5. F. Rowell, J. Seviour, A.Y. Lim, C.G. Elumbaring-Salazar, J. Loke, J. Ma, Detection of nitro-organic and peroxide explosives in latent fingermarks by DART- and SALDI-TOF-mass spectrometry. *Forensic Sci. Int*. 221 (2012) 84-91.

6. S. Francese, R. Bradshaw, N. Denison, An update on MALDI mass spectrometry based technology for the analysis of fingermarks - stepping into operational deployment. *Analyst*. 142 (2017) 2518-2546.

7. G. Groeneveld, M. de Puit, S. Bleay, R. Bradshaw, S.Francese, Detection and mapping of illicit drugs and their metabolites in fingermarks by MALDI MS and compatibility with forensic techniques. *Sci. Rep*. 5 (2015) 11716.

8. A. Banas, K. Banas, M. B. H. Breese, J. Loke, B. Heng Teo and S. K. Lim, Detection of microscopic particles present as contaminants in latent fingerprints by means of synchrotron radiation-based Fourier transform infra-red micro-imaging. *Analyst* 137 (2012) 3459-3465.

9. A. Grant, T.J. Wilkinson, D.R. Holman, M.C. Martin, Identification of Recently Handled Materials by Analysis of Latent Human Fingerprints Using Infrared Spectromicroscopy. *Appl. Spectrosc*. 59 (2005) 1182-1187.

10. Y. Mou, J.W. Rabalais, Detection and Identification of Explosive Particles in Fingerprints Using Attenuated Total Reflection-Fourier Transform Infrared Spectromicroscopy. *J. Forensic Sci*. 54 (2009) 846.

11. D.K. Williams, R.L. Schwartz, E.G Bartick, Analysis of Latent Fingerprint Deposits by Infrared Microspectroscopy. *Appl. Spectrosc*. 58 (2004) 313-316.

12. J.S. Day, H.G. Edwards, S.A. Dobrowski, A.M. Voice, The detection of drugs of abuse in fingerprints using Raman spectroscopy II: cyanoacrylate-fumed fingerprints. *Spectrochim. Acta. Part A Mol. Biomol. Spectrosc*. 60 (2004) 1725-1730.

13. J.S. Day, H.G.M. Edwards, S.A. Dobrowski, A.M. Voice, The detection of drugs of abuse in fingerprints using Raman spectroscopy I: latent fingerprints. *Spectrochim. Acta. Part A Mol. Biomol. Spectrosc*. 60 (2004) 563-568.

14. C. Cheng, T. Kirkbride, D. Batchelder, R. Lacey, T. Sheldon, In Situ Detection and Identification of Trace Explosives by Raman Microscopy BT - In Situ Detection and Identification of Trace Explosives by Raman Microscopy. (1995).

15. M.J. West, M.J. Went, The spectroscopic detection of exogenous material in fingerprints after development with powders and recovery with adhesive lifters. *Forensic Sci. Int*. 174 (2008) 1-5.

16. A. Tripathi, E.D. Emmons, P.G. Wilcox, Semi-Automated Detection of Trace Explosives in Fingerprints on Strongly Interfering Surfaces with Raman Chemical Imaging. *Appl. Spectrosc*. 65 (2011) 611-619.

17. C. Ricci, N. Curum, K.L. Andrew Chan, S. Jickells, S. Kazarian, P. Phiriyavityopas, Chemical Imaging of Latent Fingerprint Residues. *Appl Spectrosc*. 61 (2007) 514-522.

18. P.H.R. Ng, S. Walker, M. Tahtouh, B. Reedy, Detection of illicit substances in fingerprints by infrared spectral imaging. *Anal. Bioanal. Chem*. 394 (2009) 2039.

19. N.J. Crane, E.G. Bartick, R.S. Perlman, S. Huffman, Infrared spectroscopic imaging for noninvasive detection of latent fingerprints. *J. Forensic Sci*. 52 (2007) 48-53.

20. M. Tahtouh, P. Despland, R. Shimmon, J.R. Kalman, B.J. Reedy, The Application of Infrared Chemical Imaging to the Detection and Enhancement of Latent Fingerprints: Method Optimization and Further Findings. *J. Forensic Sci*. 52 (2007) 1089-1096.

21. G. Payne, C. Wallace, B. Reedy., Visible and near-infrared chemical imaging methods for the analysis of selected forensic samples. *Talanta*. 67 (2005) 334-344.

22. E. Bartick, R. Schwartz, R. Bhargava, M. Schaeberle, D. Fernandez, I. Levin, Spectrochemical analysis and hyperspectral imaging of latent fingerprints. In: *16th Meeting of the International Association of Forensic Sciences*. International Association of Forensic Sciences. (2002) 61-64.

23. A.G. Mercado, J.A. Janni, B. Gilbert, Image analysis of explosives fingerprint contamination using a Raman imaging spectrometer. *Proc SPIE*. 2511 (1995) 142-152.

24. C. Ricci, S. Bleay, S.G. Kazarian, Spectroscopic Imaging of Latent Fingermarks Collected with the Aid of a Gelatin Tape. *Anal. Chem*. 79 (2007) 5771-5776.

25. K.A. Mountfort, H. Bronstein, N. Archer, S.M. Jickells, Identification of oxidation products of squalene in solution and in latent fingerprints by ESI-MS and LC/APCI-MS. *Anal. Chem*. 79 (2007) 2650-2657.

26. G.M. Mong, C.E. Petersen, T.R.W. Clauss, Advanced Fingerprint Analysis Project - Fingerprint Constituents. Vol PNNL-13019. Pacific Northwest National Laboratory: U.S. Department of Energy. (1999).

27. N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells, Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface. *Forensic Sci. Int*. 154 (2005) 224-239.

28. R.S. Croxton, M.G. Baron, D. Butler, T. Kent, V.G. Sears, Variation in amino acid and lipid composition of latent fingerprints. *Forensic Sci. Int*. 199 (2010) 93-102.

29. R. Ramotowski, *Composition of Latent Print Residues, in: Advanced in Fingerprint Technology, 2nd Ed.* (Lee. H, Gaensslen. R, eds.). London: CRC Press (2001).

30. A. Srivastava, R. Prasad, Triglycerides-based diesel fuels. *Renew. Sust. Energy Rev*. 4 (2000) 111-113.

31. B.B. Dent, S.L. Forbes, B.H. Stuart, Review of human decomposition processes in soil. *Environ. Geol*. 45 (2004) 576-585.

32. R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese, Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids. *Rapid Commun. Mass Spectrom*. 23 (2009) 3031-3039.

33. C. Weyermann, C. Roux, C. Champod, Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis. *J. Forensic Sci*. 56 (2011) 102-108.

34. L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms. *Chem. Phys. Lipids*. 164 (2011) 457-468.

35. S.K. Kim, W.W. Nawar, Oxidative interactions of cholesterol with triacylglycerols. *J. Am. Oil Chem. Soc*. 68 (1991) 931-934.

36. A. Girod, R. Ramotowski, C. Weyermann, Composition of fingermark residue: A qualitative and quantitative review. *Forensic Sci. Int*. 223 (2012) 10-24.

37. M.V. Buchanan, K.G. Asano, A. Bohanon, Chemical Characterization of fingerprints from adults and children. *Int. Soc. Opt. Eng*. 2941 (1996) 89-95.

38. D.K. Williams, C.J. Brown, J. Bruker, Characterization of children's latent fingerprint residues by infrared microspectroscopy: Forensic implications. *Forensic Sci. Int*. 206 (2011) 161-165.

39. D. Noble, Vanished into thin air. The search for children's fingerprints. *Anal. Chem*. 67 (1995) 435A-438A.

40. K.M. Antoine, S. Mortazavi, L.M. Miller, Chemical differences are observed in children's versus adults' latent fingerprints as a function of time. *J. Forensic Sci*. 55 (2010) 513-518.

41. M.J. Bailey, N.J. Bright, S. Hinder, Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spec. *Anal. Chem*. 84 (2012) 8514-8523.

42. S. Cadd, M. Islam, P. Manson, S. Bleay, Fingerprint composition and aging: A literature review. *Sci. Justice*. 55 (2015) 219-238.

43. A. Girod, L. Xiao, B. Reedy, C. Roux, C. Weyermann, Fingermark initial composition and aging using Fourier transform infrared microscopy (μ-FTIR). *Forensic Sci. Int*. 254 (2015) 185-196.

44. F. Cuthbertson, J.R. Morris, *The Chemistry of Fingerprints, memorandum332*; (1972).

45. P. Fritz, W. van Bronswjik, K. Lepkova, Infrared microscopy studies of the chemical composition of latent fingermark residues. *Microchem. J*. 111 (2013) 40-46.

46. M. Picardo, C. Zompetta, C. De Luca, Squalene peroxides may contribute to ultraviolet light-induced immunological effects. *Photodermatol Photoimmunol Photomed*. 8 (1991) 105-110.

47. A. Johnston, K.D. Rogers, The Effect of Moderate Temperatures on Latent Fingerprint Chemistry. *Appl. Spectrosc*. 71 (2017) 2102-2110

CHAPTER 8. DISCUSSION

8.1 Implications and impact

The overarching aim of this body of work was to investigate the dynamic nature of latent fingermark chemistry. Additionally, the content within this thesis strived to, where possible, progress from academic research into fingermark chemistry to research with genuine practical applications for forensic science. Figure 45 shows how each paper develops from academic knowledge toward practical information for the forensic community.

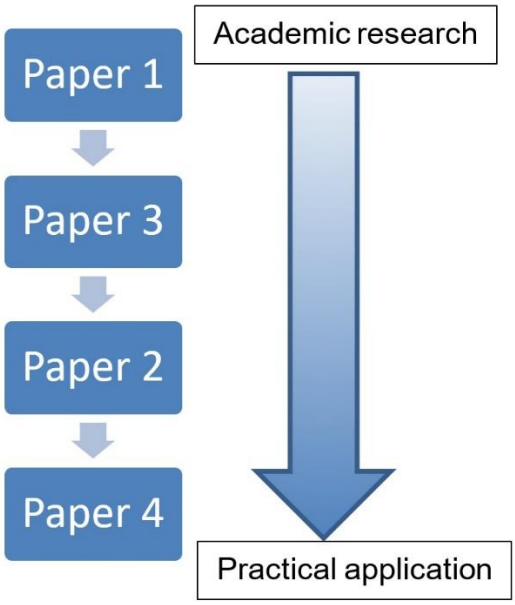


Figure 45: The development of papers from academic research to practical forensic relevance

An understanding of the applications and limitations of FTIR spectroscopy was vital before any further research could be achieved. FTIR spectroscopy has been used within forensic science in one form or another for almost 40 years, yet the majority of research into latent fingermarks has been undertaken by MS and its derivatives. As outlined in paper 1, however, FTIR spectroscopy can provide a rapid, non-destructive, holistic approach to the analysis of fingermark chemistry, capable of analysing the relationship between the various functional groups and the total organic composition (represented by the antisymmetric and symmetric

C-H stretch of CH_2 groups), for example. Unlike GC-MS, a latent fingermark can be viewed as a complete system using FTIR spectroscopy, and the relationship between specific molecular groups within that system can be analysed and chemically mapped, thus not only providing chemical analysis of the sample but also spatial information about the fingermark. GC-MS can quantifiably measure specific compounds within a fingermark and has been instrumental in developing our understanding of latent fingermark chemistry, albeit with time-consuming sample preparation and destruction of the sample. Within the context of specific molecular identification this is not possible using FTIR spectroscopy, and, as in paper 1, cosmetic contaminants, similar in composition to natural sebaceous secretions, can distort the IR spectra and could produce misleading results, whereas GC-MS can identify these contaminants and factor them into any analysis.

FTIR spectroscopy, therefore, appeared to be ideally suited for the analysis of latent fingermark chemistry as a whole and investigating the molecular interactions that take place within a fingermark post-deposition. Paper 1 outlined the capabilities of FTIR spectroscopy and dictated the areas of research that would ultimately lead to paper 4 and the investigation of contaminant degradation within latent fingermarks. As mentioned previously understanding the limitations of FTIR spectroscopy was essential to the author in directing further research, however, there is a wider impact to this work as well. Published research has investigated the various analytical techniques applied to the analysis of latent fingermark chemistry [1-12], but a direct comparison between FTIR spectroscopy and GC-MS as reported in paper 1 had only been carried out by Bailey et al [1], amongst other analytical techniques. Information within this paper could be important for crime scene investigators and forensic laboratories as it could contribute to procedural change as to how fingermarks are analysed in the future to maximize evidence retrieval. Alternatively, a summary of this work could be incorporated into an operating guide or decision making tool advising on how best to process and analyse fingermark evidence. It is not uncommon for manufacturers to make exaggerated claims regarding the capabilities of their

analytical tool, which can be misleading for a new user, especially when acquiring an unfamiliar technology. The research in paper 1 could contribute to this decision-making process, and be used as part of an independent ‘buyers’ guide' to the two orthogonal technologies, and their appropriateness for requirements.

Fingermark degradation is arguably the most active area of fingermark research currently, because it has the potential to impact on obtaining critical biometric information on an individual, and perhaps more importantly, for establishing time since deposition of that fingermark. Establishing when a fingermark was deposited would potentially enable a suspect to be ruled in or out of an investigation. By analysis of the known mechanisms of degradation, there is the potential to develop a ‘model of fingermark aging’ which could be used to indicate the time since deposition of a latent fingermark. Degradation mechanisms such as; oxidation of squalene (SQ) to SQ hydroperoxide intermediates, the presence of the known SQ oxidation end products, hexanedioic acid or pentanedioic acid, a high concentration of saturated fatty acids and low concentration of triglycerides and unsaturated fatty acids would all be integral to developing this model in adults. There has been significant research into this area of fingermark chemistry, but there remain gaps in our understanding that have prevented the development of such a model. Although certain molecular degradation products have been identified (or at least proposed) within latent fingermarks [11,13-31], the fundamental cause of these degradation pathways, i.e. the intermolecular interactions between the molecular species, is poorly understood. This is primarily because studying the behaviour and impact of a single compound, cholesterol, for example, is almost impossible within the complex matrix of components that make up a latent fingermark. It is because of this, and an understanding of the capabilities of FTIR spectroscopy derived from paper 1, that the research for paper 3 was developed.

Arguably, the only way to gain an understanding of the influence of a particular molecular component on the entire chemical system within a latent fingermark is to develop a simplified analogue. Such as presented in paper 3.

FTIR spectroscopy (in particular mid-range FTIR) is ideally suited to this type of analysis. Through peak area calculations of common functional groups, it was possible to study a whole chemical system, and, by creating simplified analogue samples that represent the basic chemical makeup of a latent fingermark, that system can be controlled and modified. The results reported in paper 3 demonstrated that two key molecules, squalene and cholesterol, are instrumental in influencing the intermolecular interactions within the analogue samples. As stated in paper 3, caution must be observed when comparing these simplified solutions to natural fingermarks. However, this data does support previous findings on the influence of these two compounds on key degradation processes [11, 24, 29, 32], and hence, for the first time, a suggested molecular interaction pathway was proposed (figure 46).

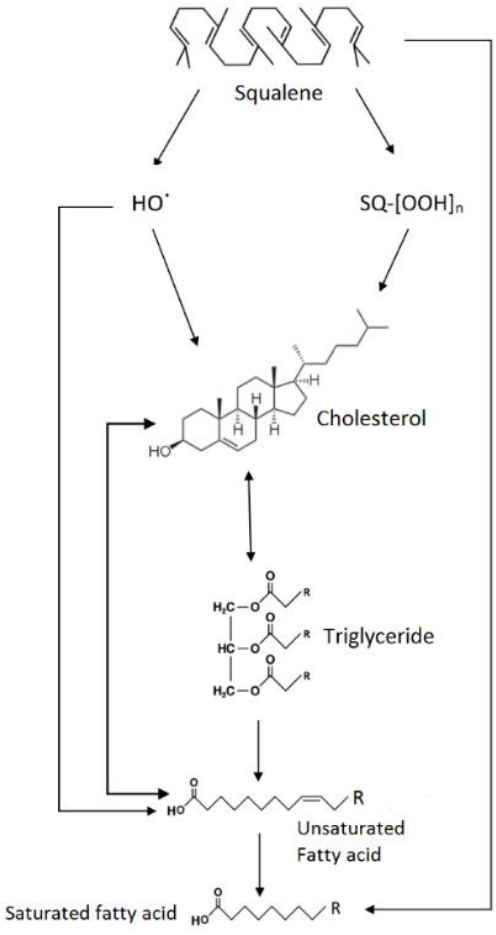


Figure 46: Proposed molecular interaction pathway of key fingermark components post-deposition

This paper aimed to provide a focus for further research into the critical intermolecular interactions that contribute to latent fingermark degradation in a way that is not possible when studying natural fingermarks. It is not possible to completely remove squalene from a natural fingermark, for example, and examine the impact on degradation processes. So although these analogue solutions, no matter how complex, will always be simplified representations of natural fingermarks, they can provide indicators into the processes that may be occurring within a natural fingermark system. Research into fingermark degradation can then be built around these indicators. For example, children's fingermarks contain low levels of squalene and triglycerides; so does cholesterol degradation occur at the same rate and to the same extent in children's fingermarks as in adults? Do the free fatty acids in children's fingermarks remain in higher concentrations than that of adults because there are fewer triglyceride or squalene degradation products to interact with, and as a result, these free fatty acids evaporate more rapidly? Questions such as these are vital in advancing our understanding of fingermark degradation chemistry in both adults and children, and simplified models can guide these investigations. Currently the vast majority studies into fingermark degradation chemistry are limited to areas of academic interest and developing a broader understanding of the field. The research is still a significant way off the ultimate goal of enabling the development of a robust model of degradation that can be applied to crime scene investigations. If such a model can be developed, which accounts for the broad inter- and intra-variability within fingermark chemistry, and capable of providing an accurate estimation of time since deposition for a fingermark found at a crime scene (that is admissible in a court of law), then it is likely that this will only happen with the integration of all avenues of research into fingermark chemistry, previous and on-going. This would include the continuing work on the identification of degradation products from various key chemical components using an array of analytical techniques, studies using extremely large sample groups looking into the intra- and inter-variability within fingermark chemistry, and the development of simplified models that can then be applied to natural fingermark behaviour. Also, the development of increasingly complex analogue solutions that behave more like natural

fingermarks, a current issue with synthetic solutions, will be a strong indication of a more comprehensive understanding of natural fingermark chemistry post-deposition.

Papers 1 and 3 were essential research projects for the author that provided an advanced understanding of the capabilities of FTIR spectromicroscopy, and an understanding of latent fingermark composition and degradation. An objective of the overall body of work, however, was to take this knowledge and apply it to more practical applications for FTIR spectromicroscopy that could have a more immediate impact on forensic investigations requiring fingermark analysis. Paper 2 investigated the effects of moderate temperature variations on latent fingermark chemistry. This research addressed a gap in the literature on the impact of moderate temperatures on latent fingermark chemistry over a short period of time. The vast majority of the published data looked at exposure to extreme temperatures, such as fires, IED detonations, and gunshot propellant [27, 28, 33-36].

A latent fingermark is not typically deposited in a static environment, and will experience (sometimes dramatic) variations in diurnal temperatures. In the UK, temperatures at certain crime scenes, typically enclosed spaces with no ventilation, inside vehicles for example, can routinely vary from 0^{o}C to 38^{o}C as reported by the London Metropolitan Police, and in more arid or tropical climates, such as southern Europe, or the Middle East (where the UK is currently providing forensic training programmes to countries such as Afghanistan and Iraq), temperatures at crime scenes have exceeded 55^{o}C. Paper 2 demonstrated that a significant reduction in fingermark composition could be observed at temperatures above 55^{o}C, and, unsurprisingly, higher temperatures saw a more rapid and complete reduction in composition. With temperatures below 55^{o}C fingermark chemistry was relatively stable within the first 5 hours post-deposition, although whatever the temperature there was evidence that oxidation processes had already begun within this timeframe (figure 47).

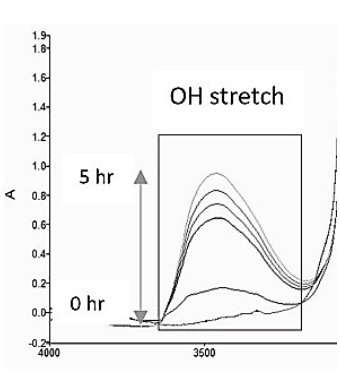


Figure 47: Evidence of oxidation processes taking place over the first 5 hours post-deposition of a latent fingermark

These results could help CSI's prioritise evidence collection and, in extreme environments, remove latent fingermarks from the crime scene to a more stable environment for analysis, if for nothing else to preserve the ridge patterns for development. This work could become even more relevant if, as discussed previously, a model for fingermark degradation to establish time since deposition can be developed and utilised for crime scene analysis. Variations in environmental temperature will likely have a significant effect on the oxidation processes within a latent fingermark, as reported in paper 2, and this would have to be factored into any model that accurately estimates time since deposition. This paper exemplifies the complexities involved in developing such a reliable model that can be used by forensic and law enforcement agencies, and how much further work is required in this area.

Paper 4 was designed to be of immediate use to the forensic community, and to be the culmination of the knowledge gained from papers 1-3. The detection of exogenous, typically illicit, particulate at a crime scene is of obvious benefit to crime scene investigators, and the detection of these compounds within latent fingermarks can narrow an investigation to particular persons of interest. Currently, the detection of narcotics or even explosive precursors, if done at all, is done by variations on mass spectrometry; extremely reliable, but usually labourious, and ultimately requires the destruction of the sample. Non-destructive techniques such as Raman and FTIR spectroscopy have been shown capable of

detecting various explosives/precursors and narcotic particulate [37-44], but are not commonly utilised as part of a forensic investigation SOP.

There were two issues with the research papers referenced in paper 4 which were worthy of address. Firstly, the choice of explosive particulate to be analysed in these papers were typically military grade explosives, such as TNT, RDX and PETN (although ammonium nitrate was also investigated). These types of explosive are no longer routinely used by terrorist organisations. These compounds, commonly used by terrorist groups in the post-war era until the turn of the century, Semtex (RDX & PETN) used in the Lockerbie bombing of 1988 for example, have passed out of favour with terrorist groups primarily due to a lack of access, a global tightening of security regarding these explosive compounds, and their complexity of synthesis. Terrorist groups now, almost universally, opt for 'homemade' explosive mixtures, IED's, synthesised from domestically available products which are almost impossible to monitor by the authorities, and are easy to make with instructions available on the internet. For this reason, the author selected two common IED precursors, ammonium nitrate, and sodium chlorate, both still commonly found in use by terrorist cells around the world.

The second issue the author aimed to address was that in previous research the contaminated fingermarks were analysed immediately after deposition. This is unrealistic concerning a criminal investigation; a crime scene, and any fingermarks within it, may not be identified by police for days or even weeks after an incident. The recently failed detonation of an IED at Parson's Green, London, is an example, the attack took place on the 15th September 2017, but forensic officers were establishing crime scenes and lifting aged fingermarks 14 days later. Therefore, as discussed previously, those fingermarks of interest were likely to have begun to degrade. Any interactions between the exogenous particulate within the fingermarks and the degrading endogenous components could have an impact on their detectability over time.

Paper 4 demonstrated that, after 30 days, all three contaminants analysed (ammonium nitrate, sodium chlorate, and cocaine) were detected (figure 48). There had been no significant degradation evident for sodium chlorate and cocaine, however, ammonium nitrate showed a 31% decrease in absorbance (and therefore quantity) over the first 10 days. This demonstrates that ammonium nitrate is prone to a degree of degradation, but perhaps more significantly, indicates that degradation of certain contaminants may well occur within the first 10 days' post-deposition when the water content of a fingermark is at its highest and the oxidation of unsaturated lipids will be most active.

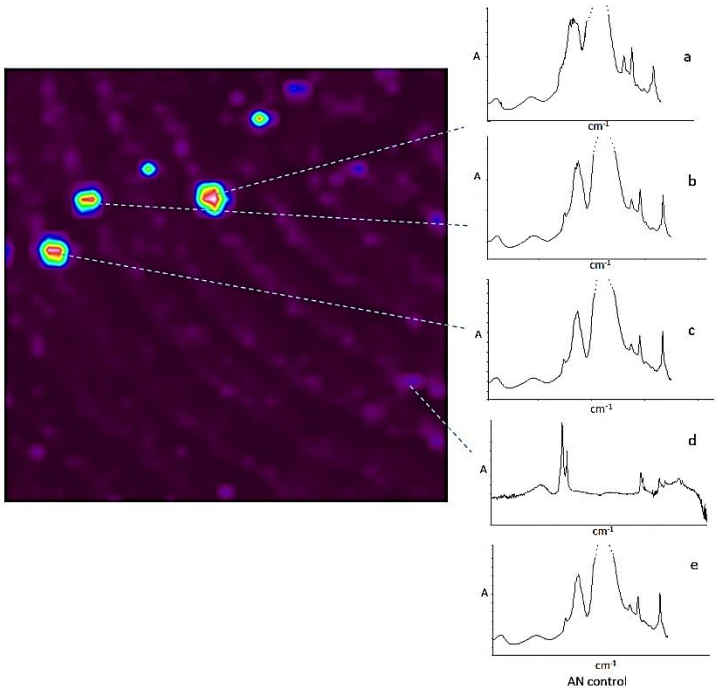


Figure 48: The detection of ammonium nitrate in a latent fingermark

This research, therefore, has significant implications for law enforcement agencies. Not only does it demonstrate the effective use of FTIR spectromicroscopy in non-destructively identifying, and chemically mapping, illicit compounds weeks after deposition, but it shows that certain compounds of interest, considered chemically stable, may degrade faster than others, and should be prioritised where possible during an investigation.

This body of work has demonstrated the effectiveness of FTIR spectroscopy as a tool for academic research into fingermark chemistry, and as practical tool that can be utilised by law enforcement agencies. The principal advantage of FTIR spectroscopy is the rapid, non-destructive analysis of latent fingermarks, both in terms of immediate benefit to forensic investigators, i.e. illicit substance detection, and the potential long-term applications, i.e. biometric analysis of fingermark chemistry, and temporal modelling.

The non-destructive nature and rapid analysis of FTIR spectroscopy can be utilised as a decision-making tool within a crime scene investigation, providing CSI's and investigating officers with the capability to rapidly analyse, and chemically map fingermarks (for both illicit substance detection and to provide a ridge pattern for suspect identification). The results of which provide information that could aid decision making and direct a line of enquiry.

Future advancements in technology could conceivably see analysis being even faster through the development of portable IR spectroscopic detectors designed for fingermark analysis, allowing for presumptive sampling at the crime scene. This is not a near future capability and would require significant research and development (R&D) to achieve a workable Technology Readiness Level (TRL), but the component parts of such a device are already in existence, such as micro-staring array detectors, portable imaging ATR technologies and linear micro-scanners. With such a technology it is conceivable that fingermarks could be lifted from a crime scene on a bespoke substrate and placed onto a portable or vehicle-based IR detector. The fingermark would then be analysed for lipid content, illicit substances, and chemically mapped, obtaining images of ridge patterns that could be uploaded to the national fingermark database. The pathway of development could be envisaged in much the same way as explosive detectors have evolved in the post-9/11 era (figure 49).

Figure 49: Thermo Fisher Scientific portable FTIR explosives/narcotics detector

Until approximately 2003/2004 trace explosive detection and analysis was exclusively the realm of laboratory analytics. Field samples would be taken back to an authorised laboratory for analysis, typically using benchtop GC-MS or IMS, which could take up to 12 hours to process. Post-9/11 however, and the invasion of Iraq in 2003 by the U.S. and its allies, the need for compact, portable explosive detectors, for use in the field, increased significantly. Since 2004 the explosives detection market has been inundated with various technologies, including portable IR and Raman detectors, for the field detection of trace and bulk explosives. These detectors are treated as presumptive tests by first responders, and confirmation is always sought by conventional laboratory analysis where available. These portable technologies have undoubtedly helped the response process for both military operations in Iraq and Afghanistan, and more recently assisted police decision making and responses in domestic terrorism incidents. It is perfectly conceivable that this approach could be applied to the development of fingermark analysis in the field once the benefits have been demonstrated, thus creating demand.

A more immediate benefit of FTIR spectromicroscopy, and in particular, spectroscopic imaging is as admissible evidence in court. Forensic science is currently under considerable pressure, both in the UK and U.S., to justify itself in terms of analytical techniques, interpretation of data and conclusions drawn.

The "misuse and misunderstanding of forensic evidence" (Hugh Miller. Traces of Guilt: Forensic Science Under the Microscope) has led to a number of miscarriages of justice in recent years, and some cases have led to a wide-scale review of forensic practices. Most recently in the UK for example, two forensic scientists were arrested for manipulating hundreds of drugs tests in a forensic laboratory in Manchester in 2017. In the U.S. a case was thrown out of court because the complexity of the analytical technique used was “well beyond the understanding of even a reasonably educated U.S. citizen” according to the presiding judge.

The scientific principles of most analytical techniques, including FTIR spectroscopy, require a relatively advanced level of scientific understanding, and the presentation of any results from these techniques in court is usually reliant on the jury understanding the basic principles of that technique. Presenting a GC-MS chromatogram to identify the presence of a narcotic or explosive for example, requires a complex explanation of the peaks present and how they relate to chemical composition of the analyte. Presenting that same information using a spectroscopic image, however (paper 4), that looks like a fingermark, with an identifiable ridge pattern, and the exogenous particles of interest visible, has far more of a visual impact, and is more accessible to a jury with varying scientific backgrounds.

FTIR spectroscopy is now considered an established forensic tool for the analysis of latent fingermarks, but its use is primarily focused on academic research and the advancement of knowledge as opposed to a technique routinely used by practising forensic examiners applied to criminal investigations. Currently the chemical analysis of latent fingermarks to detect trace amounts of illicit substances are not often (if ever) carried out by CSI's, and certainly, our understanding of latent fingermark chemical composition is not yet sufficient to be utilised by law enforcement agencies for determining much biometric information. The work presented here has demonstrated the ability of FTIR spectromicroscopy to not only advance our academic understanding of latent fingermark chemistry, but also provided research of genuine and immediate use

to the forensic community. This work can now be expanded upon and builds a case for the use of FTIR spectromicroscopy as an essential tool for criminal investigations.

8.2 References

1. M.J. Bailey et al, Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spec., and attenuated total reflection Fourier Transform Infrared Spectroscopic Imaging: An Intercomparison. *Anal. Chem*. 84 (2012) 8514-8523.

2. C. Weyermann, C. Roux, C. Champod, Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis. *J. Forensic Sci*. 56 (2011) 102-108.

3. R. Ramotowski, *Composition of Latent Print Residues, in: Advanced in Fingerprint Technology, 2nd Ed.* (Lee. H, Gaensslen. R, eds.). London: CRC Press (2001).

4. A. Koenig, A. Girod, C. Weyermann, Identification of wax esters in fingermark residues by GC/MS and their potential use as aging parameters. *J. Forensic Ident*. 61(6) (2011) 652-76.

5. S. Francese, R. Bradshaw, L.S. Ferguson, R. Wolstenholme, M.R. Clench, S. Bleay, Beyond the ridge pattern: Multi-informative analysis of latent fingermarks by MALDI mass spectrometry. *Analyst*. 138 (15) (2013) 4215-4228.

6. L. Ferguson, R. Bradshaw, R. Wolstenholme, M. Clench, S. Francese, Two-Step Matrix Application for the Enhancement and Imaging of Latent Fingermarks. *Anal. Chem*. 83 (14) (2011) 5585–5591.

7. R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese, Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids. *Rapid Commun. Mass Spectrom*. 23 (2009) 3031-3039.

8. R. Bradshaw, W. Rao, R. Wolstenholme, M.R. Clench, S. Bleay, S. Francese, Separation of overlapping fingermarks by Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging. *Forensic science international*. 222 (1–3) (2012) 111-124

9. R. Bradshaw, S. Bleay, R. Wolstenholme, M.R. Clench, S. Francese, Towards the integration of matrix assisted laser desorption ionisation mass spectrometry imaging into the current fingermark examination workflow. *Forensic Sci Int*. 232 (2013) 111-124.

10. B. Emerson, B. Durham, J. Gidden, J.O. Lay, Laser desorption/ionization time-of-flight mass spectrometry of triacylglycerols and other components in fingermark samples. *J Forensic Sci*. 56 (2) (2011) 381-389.

11. K.A. Mountfort, H. Bronstein, N. Archer, S.M. Jickells, Identification of oxidation products of squalene in solution and in latent fingerprints by ESI-MS and LC/APCI-MS. *Anal. Chem*. 79 (2007) 2650-2657.

12. A.Y. Lim, J. Ma, Z. Ma, F. Rowell, Separation of fingerprint constituents using magnetic silica nanoparticles and direct on-particle SALDI-TOF-mass spectrometry. *Journal of Chromatography B.* 879 (23) (2011) 2244–2250.

13. S. Cadd, M. Islam, P. Manson, S. Bleay, Fingerprint composition and aging: A literature review. *Sci. Justice*. 55 (2015) 219-238.

14. A. Girod, L. Xiao, B. Reedy, C. Roux, C. Weyermann, Fingermark initial composition and aging using Fourier transform infrared microscopy (μ-FTIR). *Forensic Sci. Int*. 254 (2015) 185-196.

15. F. Cuthbertson, J.R. Morris, *The Chemistry of Fingerprints, memorandum332*; (1972).

16. N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells, Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface. *Forensic Sci. Int*. 154 (2005) 224-239.

17. R.S. Croxton, M.G. Baron, D. Butler, T. Kent, V.G. Sears, Variation in amino acid and lipid composition of latent fingerprints. *Forensic Sci. Int*. 199 (2010) 93-102.

18. A. Girod, R. Ramotowski, C. Weyermann, Composition of fingermark residue: A qualitative and quantitative review. *Forensic Sci. Int*. 223 (2012) 10-24.

19. P. Fritz, W. van Bronswjik, K. Lepkova, Infrared microscopy studies of the chemical composition of latent fingermark residues. *Microchem. J*. 111 (2013) 40-46.

20. C. Weyermann, C. Roux, C. Champod, Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis. *J. Forensic Sci*. 56 (2011) 102-108.

21. G.M. Mong, C.E. Petersen, T.R.W. Clauss, Advanced Fingerprint Analysis Project - Fingerprint Constituents. Vol PNNL-13019. Pacific Northwest National Laboratory: U.S. Department of Energy. (1999).

22. M. Picardo, C. Zompetta, C. De Luca, Squalene peroxides may contribute to ultraviolet light-induced immunological effects. *Photodermatol Photoimmunol Photomed*. 8 (1991) 105-110.

23. A. Srivastava, R. Prasad, Triglycerides-based diesel fuels. *Renew. Sust. Energy Rev*. 4 (2000) 111-113.

24. L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms. *Chem. Phys. Lipids*. 164 (2011) 457-468.

25. T. Atherton, R. Croxton, M. Baron, J. Gonzalez-Rodriguez, L. Gámiz-Gracia, A.M. García-Campańa. Analysis of amino acids in latent fingerprint residue by capillary electrophoresis-mass spectrometry. *J. Sep Sci*. 2012. 35:2994-2999.

26. A. Stadtman, A. Cherkes, C. Anfinsen, Studies on the microbiological degradation of cholesterol, *J. Biol. Chem.* 206 (2) (1954) 511–523.

27. A. Richmond-Aylor, S. Bell, P. Callery, K. Morris. Thermal degradation analysis of amino acids in fingerprint residue by pyrolysis GC-MS to develop new latent fingerprint developing reagents. *J. Forensic Sci*. 2007. 52(2):380-382.

28. G. De Paoli, S.A. Lewis, E.L. Schuette, L.A. Lewis, R.M. Connatser, T. Farkas. Photo- and thermal-degradation studies of select eccrine fingerprint constituents. *J. Forensic Sci*. 2010. 55(4):962-969.

29. S. K. Kim and W. W. Nawar, Parameters influencing cholesterol oxidation, *Lipids*. 28 (10) (1993) 917–922.

30. R.S. Greene, D.T. Downing, P.E.Pochi, J.S. Strauss, Anatomical Variation in the Amount and Composition of Human Skin Surface Lipid. *J. Inv. Derm*. 54 (3) (1970) 240–247.

31. L. L. Smith, Cholesterol autoxidation 1981–1986, *Chem. Phys. Lipids*. 44 (2–4) (1987) 87–125.

32. S.K. Kim, W.W. Nawar, Oxidative interactions of cholesterol with triacylglycerols. *J. Am. Oil Chem. Soc*. 68 (1991) 931-934.

33. A.G. Brown, D. Sommerville, B.J Reedy, R.G Shimmon, M. Tahtouh. Revisiting the thermal development of latent fingerprints on porous surfaces: new aspects and refinements. *J. Forensic Sci*. 2009. 54(1):114-121.

34. A. Dominick, N. Nicdaeid, S. Bleay, V. Sears. The recoverability of fingerprints on paper exposed to elevated temperatures -Part 1: comparison of enhancement techniques. *J. Forensic Identif*. 2010. 59(3): 325-339.

35. C.M.A. Girelli, B.J.M. Lobo, A.G. Cunha, J.C.C. Freitas, F.G. Emmerich, Comparison of practical techniques to develop latent fingermarks on fired and unfired cartridge cases, *Forensic Sci. Int.* 250 (2015) 17–26.

36. W.W. Harper. Latent Fingerprints at High Temperatures. *J. Crim Law Criminol*. 1938. 29(4):580-583.

37. A. Grant, T.J. Wilkinson, D.R. Holman, M.C. Martin, Identification of Recently Handled Materials by Analysis of Latent Human Fingerprints Using Infrared Spectromicroscopy. *Appl. Spectrosc*. 59 (2005) 1182-1187.

38. D.K. Williams, R.L. Schwartz, E.G Bartick, Analysis of Latent Fingerprint Deposits by Infrared Microspectroscopy. *Appl. Spectrosc*. 58 (2004) 313-316

39. J.S. Day, H.G. Edwards, S.A. Dobrowski, A.M. Voice, The detection of drugs of abuse in fingerprints using Raman spectroscopy II: cyanoacrylate-fumed fingerprints. *Spectrochim. Acta. Part A Mol. Biomol. Spectrosc*. 60 (2004) 1725-1730.

40. C. Cheng, T. Kirkbride, D. Batchelder, R. Lacey, T. Sheldon, In Situ Detection and Identification of Trace Explosives by Raman Microscopy BT - In Situ Detection and Identification of Trace Explosives by Raman Microscopy. (1995).

41. M.J. West, M.J. Went, The spectroscopic detection of exogenous material in fingerprints after development with powders and recovery with adhesive lifters. *Forensic Sci. Int*. 174 (2008) 1-5.

42. A. Tripathi, E.D. Emmons, P.G. Wilcox, Semi-Automated Detection of Trace Explosives in Fingerprints on Strongly Interfering Surfaces with Raman Chemical Imaging. *Appl. Spectrosc*. 65 (2011) 611-619.

43. Y. Mou, J.W. Rabalais, Detection and Identification of Explosive Particles in Fingerprints Using Attenuated Total Reflection-Fourier Transform Infrared Spectromicroscopy. *J. Forensic Sci*. 54 (2009) 846 - 850.

44. C. Ricci, S. Bleay, S.G. Kazarian, Spectroscopic Imaging of Latent Fingermarks Collected with the Aid of a Gelatin Tape. *Anal. Chem*. 79 (2007) 5771-5776.

CHAPTER 9. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Outline

Conclusions drawn from this work are presented, in addition to recommendations for future work. Finally, an explanation of the key contributions of this research to general knowledge and specifically to the development of IR spectroscopy as a tool for criminal investigations is provided.

9.1 Research conclusions

During this work, the dynamic nature of latent fingermark chemistry was investigated.

Initially, the capabilities and limitations of FTIR spectroscopy were compared to that of GC-MS, the gold standard in the analysis of latent fingermarks. The ability of FTIR spectromicroscopy to identify specific functional groups within latent fingermarks and provide spatial maps of these functional groups was demonstrated. Although FTIR spectroscopy could not achieve the specificity of GC-MS in providing data on specific compounds, such as a specific fatty acid or wax ester, FTIR spectroscopy did allow for the analysis of a fingermark as a holistic system. Using the symmetric and asymmetric C-H stretch of CH_2 groups at 2924 cm^{-1} - 2854 cm^{-1} as the combination of all the organic material within the fingermark it was possible to compare that to specific functional groups and study their relationship, something not possible using GC-MS analysis. This investigation did demonstrate how FTIR spectra could be distorted by lipid-based cosmetics such as moisturisers which will contribute to the symmetric and asymmetric C-H stretch of CH_2 groups and potentially give a false representation of the natural lipid content of a fingermark. GC-MS, due to its specificity, was capable of identifying such exogenous compounds, even those with similar chemical structures to endogenous lipids.

Paper 1 provided the basic understanding and groundwork for the following research looking into the effects of moderate temperature variations on latent

fingermark chemistry. The versatility of FTIR spectromicroscopy enabled *in situ* sampling while the sample was maintained at the required temperature. This allowed for changes in fingermark chemistry to be observed in real time through spectral analysis. Pure compounds such as squalene were also heated to identity any trends in decomposition that were comparable to those observed within the natural fingermarks. The study demonstrated that fingermarks heated to 55^{o}C and above showed a significant reduction in the quantity of lipid components over the 5-hour period, but fingermarks heated to 45^{o}C and below showed little change in composition within the same period. Arguably the key finding from this study was the identification of an increase in the OH stretch region (3250 cm^{-1}) of all the fingermarks analysed. This increase indicating that oxidation processes within fingermarks are taking place almost immediately after deposition. Although there appeared to be no relationship between time/temperature and the rate of oxidation, the quantity of the oxidation products was temperature dependent, with the higher temperatures (65^{o}C and 75^{o}C) limiting the concentration of hydroxide groups being formed. This is most likely due to the loss of water and of the more volatile organic compounds at these temperatures.

The study into moderate temperature dependent degradation of latent fingermarks developed the idea of the importance of intermolecular interactions for the degradation of fingermark composition post-deposition. Studying intermolecular interactions in natural fingermarks is near impossible due to the complex matrix of components that make up a fingermark. Removing or inhibiting one compound to study its impact on the fingermark system as a whole is not possible. Because of this a simplified analogue solution was developed, containing only the most common components, which represented all the compounds within that family. Serine, the most abundant amino acid, represented all the amino acids present within a natural fingermark for example. Additional solutions were then produced, each with a single component from the control solution removed. This allowed for the impact of this compound on the interactions with the remaining components to be investigated. FTIR spectroscopy again demonstrated its versatility in analysing fingermark chemistry

as a system and by quantifying changes in peak width, and therefore intermolecular interactions, in the absence or presence of a particular compound, thus allowing the effect of these compounds on the system to be analysed.

The data suggested that squalene and cholesterol were critical in driving intermolecular interactions within these solutions. The absence of these two compounds caused a significant reduction in IR peak width not seen in the absence of any other compounds. It was stressed that caution must be observed when drawing direct comparisons between analogue samples and natural fingermarks, as previous literature has stated, although much of this caution was related to validation trials for fingermark development reagents. Within the context of fundamental research, however, there is significant value in the use of these simplified solutions as they can identify potential avenues of research that would otherwise be impossible.

This research not only identified the potential importance of squalene and cholesterol in intermolecular interaction pathways within fingermarks but also provided an additional explanation for the behaviour of children's fingermarks. The lack of squalene and triglycerides in children's fingermarks could inhibit the degradation chain, and thus lipid compounds are unable to form more stable end products. Short chain, unbranched fatty acids, typical of children's fingermarks, as well as other lipids, are therefore more susceptible to evaporation; hence children's fingermarks have been reported to ‘vanish' from a crime scene.

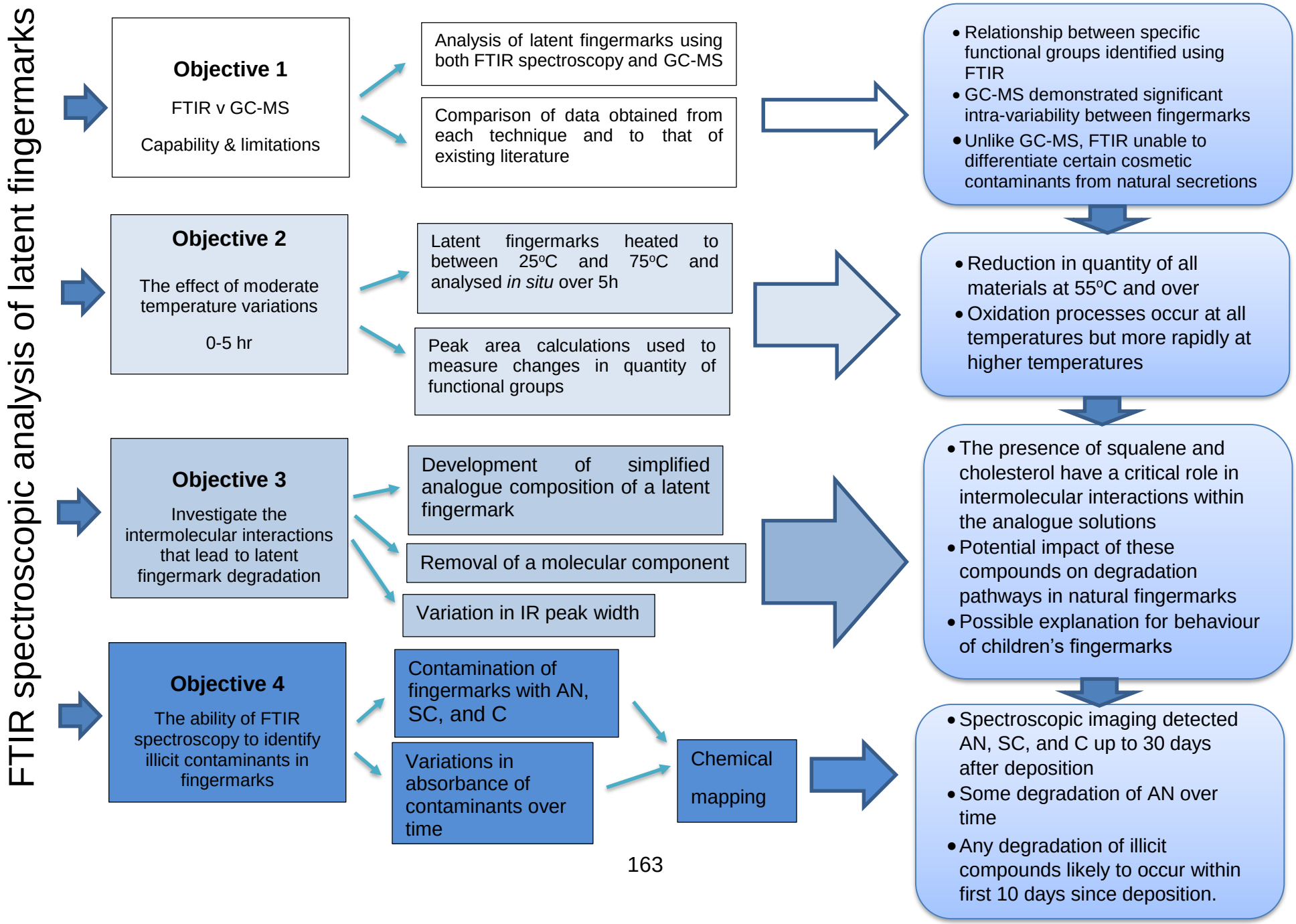
Finally, the culmination of this research was the detection of illicit compounds within latent fingermarks over time using FTIR spectroscopy and spectroscopic imaging. Previous research had demonstrated the ability of FTIR spectroscopy to chemically map and identify (typically) military grade explosives within latent fingermarks; however, these contaminated fingermarks were analysed immediately after deposition. This, however, is unrealistic during criminal investigations where fingermarks may not be discovered and collected for days or even weeks after deposition. It was important therefore to investigate the effect of natural fingermark degradation mechanisms on these contaminants over time. For the first time an understanding of fingermark chemistry and degradation,

gained from the previous studies, was combined with the ability to chemically identify and map illicit substances within latent fingermarks. The contaminants used for the study were chosen for their current relevance regarding terrorist activities and narcotics consumption in Western Europe and the US. Ammonium nitrate (AN) and sodium chlorate (SC) are common IED precursors used by terrorist cells all over the world, and cocaine (C) consumption has increased significantly within Western societies and is no longer purely a recreational drug of the wealthy.

The research in this study demonstrated that FTIR spectromicroscopy could reliably detect and identify all three contaminants when compared to a control sample and chemically map their distribution within a fingermark. Most importantly it remained possible to detect all three contaminants up to thirty days after deposition, with only a slight degradation of AN, almost entirely within the first 10 days' post-deposition. This degradation is likely due to the dynamic nature of fingermark chemistry within the first ten days. The majority of oxidation mechanisms taking place during this time period, thus unsaturated lipids would interact with the ammonium and nitrate ions, and the water content within the fingermark is at its highest making ion dissociation more likely.

The work presented in this thesis has demonstrated the capabilities of FTIR spectroscopy as a holistic analytical tool for the analysis of latent fingermarks. FTIR spectromicroscopy has shown capable of identifying key functional groups and their intra-variability, as well as mapping their distribution within the fingermark. For the first time, the versatility of FTIR spectromicroscopy allowed for the *in situ,* real-time analysis of latent fingermarks whilst subjected to varying temperatures, and the study of intermolecular interactions of analogue representations of latent fingermarks. The culmination of the work being the identification and mapping of contaminants within latent fingermarks over time. This research is unique to the capabilities of FTIR spectroscopy and spectromicroscopy and would not be possible utilising alternative analytical techniques such as GC-MS or MALDI-TOF-MS, primarily due to the destructive nature of these techniques. This study has demonstrated a role for FTIR

spectroscopy not only for the academic study of latent fingermark chemistry and behaviour, but also as a tool for forensic scientists to rapidly gather valuable forensic evidence on an individual without destroying the fingermark. As our understanding of fingermark composition and aging develops, and more reliable aging models can be generated, then FTIR spectroscopy is likely to have an even greater role for the forensic investigator. The technique could be used to rule fingermarks in or out of an investigation based on their state of chemical decomposition, to establish an age range, gender, and even time since deposition. With the miniaturisation of key technologies, this could conceivably be done as a presumptive test at the crime scene, or in a mobile laboratory, and help to guide an investigation to focus on a particular avenue of enquiry.


FTIR spectroscopic analysis of latent fingermarks
Objective 1
FTIR v GC-MS
Capability & limitations
Analysis of latent fingermarks using both FTIR spectroscopy and GC-MS
Comparison of data obtained from each technique and to that of existing literature
• Relationship between specific functional groups identified using FTIR
• GC-MS demonstrated significant intra-variability between fingermarks
• Unlike GC-MS, FTIR unable to differentiate certain cosmetic contaminants from natural secretions
Objective 2
The effect of moderate temperature variations
0-5 hr
Latent fingermarks heated to between 25ºC and 75ºC and analysed *in situ* over 5h
Peak area calculations used to measure changes in quantity of functional groups
• Reduction in quantity of all materials at 55ºC and over
• Oxidation processes occur at all temperatures but more rapidly at higher temperatures
Objective 3
Investigate the intermolecular interactions that lead to latent fingermark degradation
Development of simplified analogue composition of a latent fingermark
Removal of a molecular component
Variation in IR peak width
• The presence of squalene and cholesterol have a critical role in intermolecular interactions within the analogue solutions
• Potential impact of these compounds on degradation pathways in natural fingermarks
• Possible explanation for behaviour of children's fingermarks
Objective 4
The ability of FTIR spectroscopy to identify illicit contaminants in fingermarks
Contamination of fingermarks with AN, SC, and C
Variations in absorbance of contaminants over time
Chemical mapping
• Spectroscopic imaging detected AN, SC, and C up to 30 days after deposition
• Some degradation of AN over time
• Any degradation of illicit compounds likely to occur within first 10 days since deposition.

9.2 Contribution to knowledge

The work presented in this thesis focused on critical gaps in the literature and provided new insights into the behaviour of latent fingermarks. The results provide a significant contribution to knowledge across a wide range of disciplines within latent fingermark research.

Within the field of fingermark chemistry research, the chemical degradation of fingermarks has been, and continues to be, comprehensively studied, particularly focusing on degradation products. Research has shown that fingermark degradation is significantly affected by environmental conditions, such as light, temperature, and humidity, but little research had focused on the short-term effects of moderate temperatures on latent fingermarks. The results in this thesis demonstrate that higher moderate temperatures do have an effect on fingermark composition within five-hours of deposition, and that oxidation mechanisms occur at any moderate temperature almost immediately after deposition. This has relevance for the forensic community as these oxidation processes are likely to affect certain development techniques, and at higher temperatures forensic investigators may need to prioritise fingermark evidence for removal from the crime scene for the sake of preservation.

The process of fingermark degradation is at the forefront of latent fingermark research, a better understanding of the degradation mechanisms that take place post-deposition will have a profound effect for both research applications and criminal investigations involving fingermarks. Currently, these processes are poorly understood with only certain degradation products identified, and the mechanisms that generate those products poorly defined. Intermolecular interactions are key to these processes but have rarely been investigated due to the complexity of fingermark chemistry. The analogue solutions study presented in this thesis contribute further to this extremely complex and variable process. The identification of two key components that significantly affected intermolecular interactions within these simplified solutions points to their importance for natural fingermark degradation and may provide a focus for further research on natural

fingermarks. The research presented here suggests the significance of these two compounds for fingermark degradation and supports previous research proposing a chain of degradation and their significance within it. This research provides greater insight into this complex process and can be built upon to investigate further the importance of these compounds for oxidation processes within fingermarks.

During criminal investigations the ability to detect illicit particulate within latent fingermarks is of immediate benefit to investigating officers, this is especially true if those fingermarks can then be attributed to an individual. Paper 4 presented within this thesis demonstrates the ability for FTIR spectromicroscopy to identify and chemically map certain contaminants but, for the first time, accounts for the temporal degradation of these compounds. Accounting for the effects of temporal decomposition of a latent fingermark on these contaminants is far more realistic to an on-going criminal investigation. This was only possible with an in-depth understanding of fingermark chemistry and aging, again something not previously considered when investigating the detection of illicit substances in fingermarks.

Overall, this thesis has demonstrated previously unreported research into the behaviour of latent fingermarks, accounting for more realistic conditions such as temporal degradation and moderate variations in temperature. The research has strengthened previous work and provided compelling evidence for further avenues of research to better understand molecular interactions within fingermarks. Finally, this work has demonstrated the versatility of FTIR spectroscopy for the analysis of latent fingermarks, and how this technology could be utilised both within the laboratory and in the field. This provides a platform for future research and debate.

9.3 Future Recommendations

Various aspects of latent fingermark chemistry have been studied for several decades. The work presented in this thesis has contributed to furthering knowledge within the field, and future work, generated from this research, is a necessity if a better understanding of fingermark composition, degradation and molecular interactions are to be gained. It is also essential that future work aims to be of greater benefit to the forensic and law enforcement communities.

For this thesis, latent fingermarks were subjected to moderate temperatures, and irrespective of the temperature rapid oxidation was observed over the 5 hours post-deposition (section 5.4.1), this suggests that the activation energy for this decomposition was below 25^{o}C. FTIR spectroscopy was able to identify this oxidation taking place in real time but identifying the individual components that contributed to this process is not possible using this technique. Further research is required to identify the reactants that oxidise so quickly within a latent fingermark with greater granularity than is currently known. Such work would identify those compounds that are most likely to degrade quickly and help provide information on the time since deposition, and may potentially help the production of more effective fingermark development reagents. These compounds that oxidise so rapidly may provide a better understanding of the behaviour of children's fingermarks depending on whether they are present or absent. Further work in this area may also help forensic scientists identify and process fingermarks in more extreme environments where higher temperatures will degrade the contents of a fingermark much faster.

The development and use of synthetic fingermark solutions has historically had mixed results within the field of fingermark research, trials and validations. Solutions designed to represent natural fingermarks have only been moderately successful in validation trials and reported to have 'behaved differently' when introduced to development reagents. This lack of success in creating realistic synthetic solutions, accounting for the many hundreds of compounds found within a natural fingermark and that is adaptable to the many external factors that can

influence them, is a primary indication of the current lack of understanding of fingermark chemistry. Continued research is required to fill these gaps in the literature. Future work, possibly starting with simplified analogue solutions as in this thesis, that can more accurately replicate the behaviour of natural fingermarks, would undoubtedly better our understanding of fingermark chemistry. Aging of synthetic solutions, for example, to establish if the same degradation products are produced as in natural fingermarks would be a strong indicator of an advancement in our understanding. It would also go some way to addressing the existing scepticism around synthetic solutions and their value to the field both as a tool for fundamental research and in validation trials.

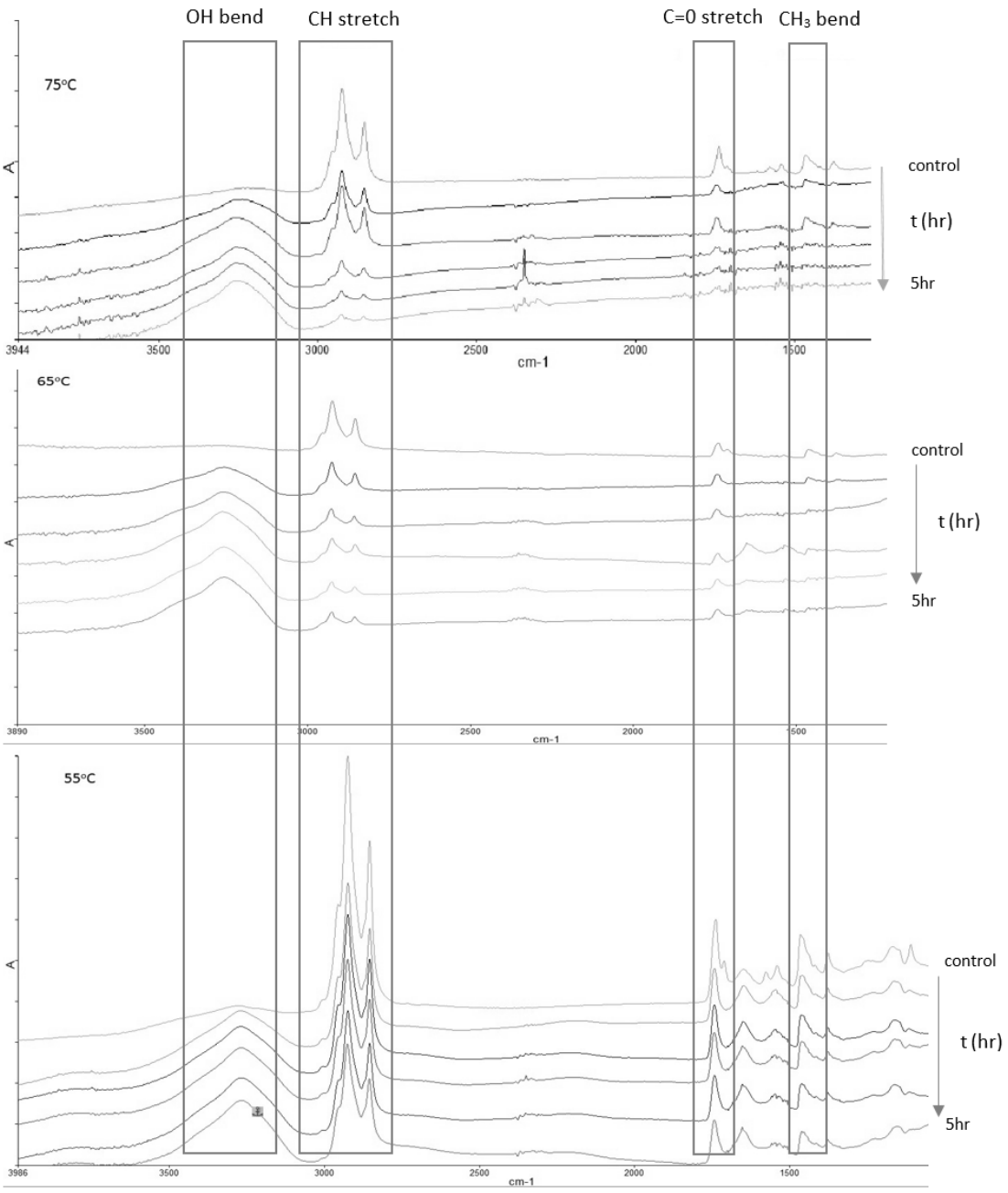
In this thesis latent fingermarks were contaminated with three exogenous compounds of particular relevance to today's law enforcement agencies. The study showed that these compounds were still detectable after thirty days within a fingermark exposed to normal diurnal light conditions and at room temperature and humidity. Only one compound (ammonium nitrate), showed any signs of degradation and that was primarily within the first ten days' post-deposition. IED's commonly used by terrorist groups are synthesised from a myriad of precursors, yet require certain basic properties to be effective, (an oxidiser, or an acidic solution for example). Future work to investigate how these categories (or even specific compounds of interest, such as acetone) interact with fingermark chemistry could be of great value to law enforcement agencies. The research may identify a bespoke reaction product present within the fingermark which indicates the handling of an illicit compound and would therefore enable the generation of a specific development reagent accordingly. Knowing if any IED precursors or narcotics degrade rapidly within fingermarks is also of use to forensic investigators, it may help prioritise analysis of fingermark evidence, or target analysis to detect a specific end product only produced in the presence of a specific contaminant.

The use of FTIR spectroscopy for the analysis latent fingermarks has been demonstrated in this thesis, all research being carried out on the same benchtop FTIR microscope. More generally IR spectroscopy has shown great potential as

a portable, handheld device for various applications including gas analysis, liquid and solid explosives detection and compound identification. Further work, utilising advancements in IR technology; micro-scanners and detectors as well as ATR technology, could enable the development of a portable handheld, or mobile benchtop FTIR fingermark analysis device, in much the same way as MALDI-MS technology is being utilised currently. These devices could be used at the crime scene or in a mobile laboratory to rapidly, and non-destructively analyse fingermarks and provide immediate presumptive information to the investigating officers.

Appendix A : Full spectral and graphical data from paper 2

A.1 Spectral data



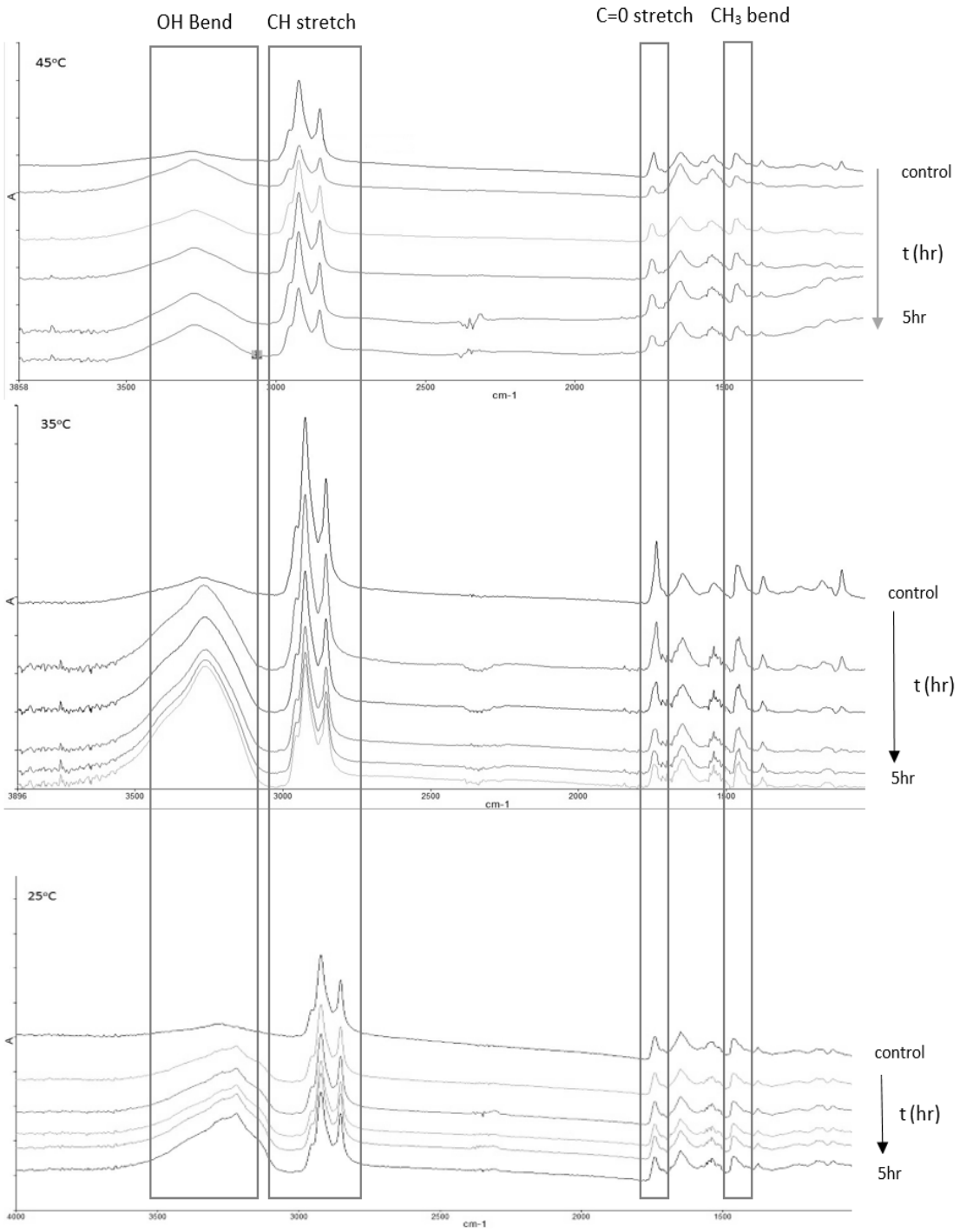
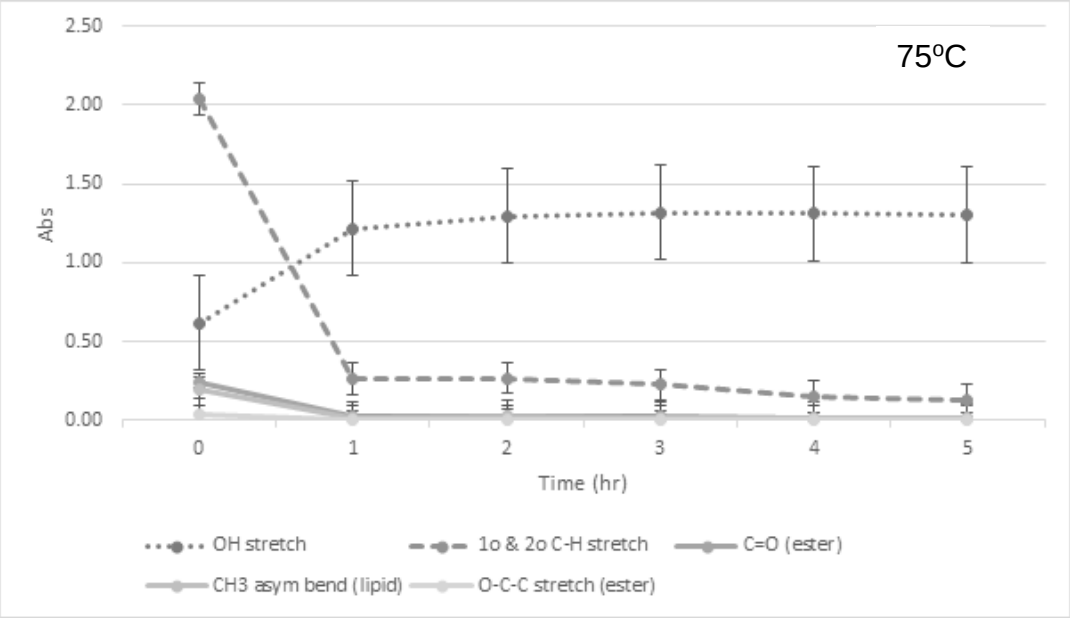
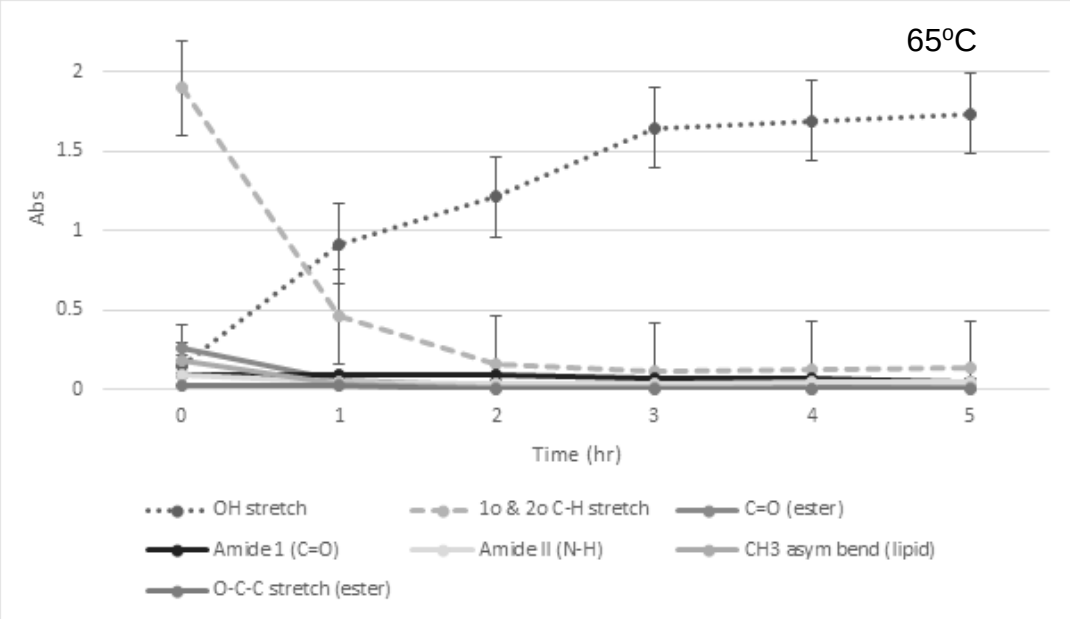
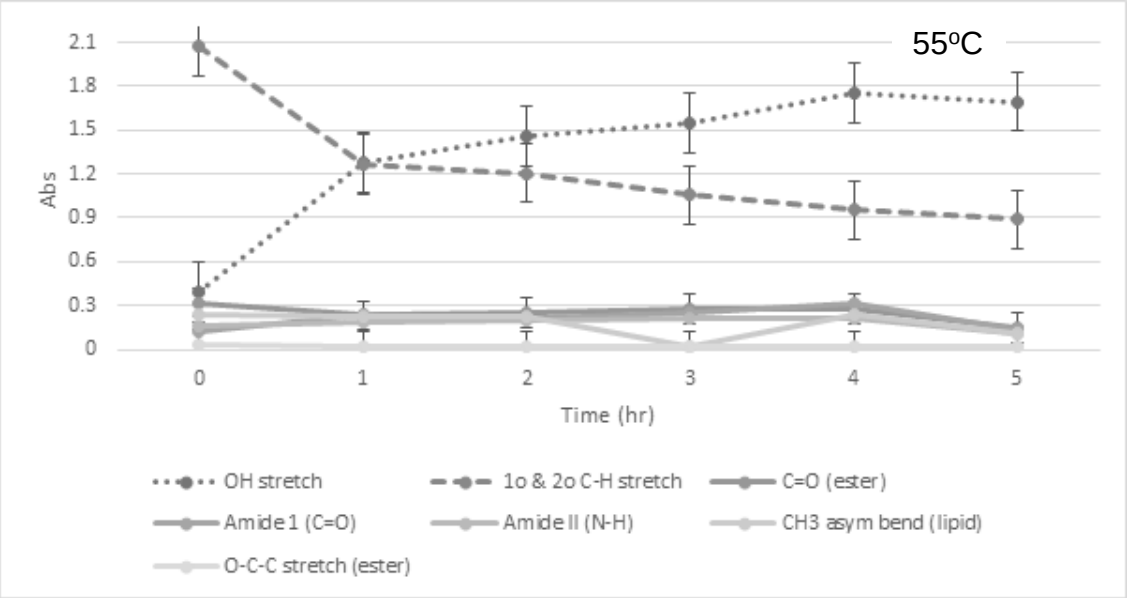


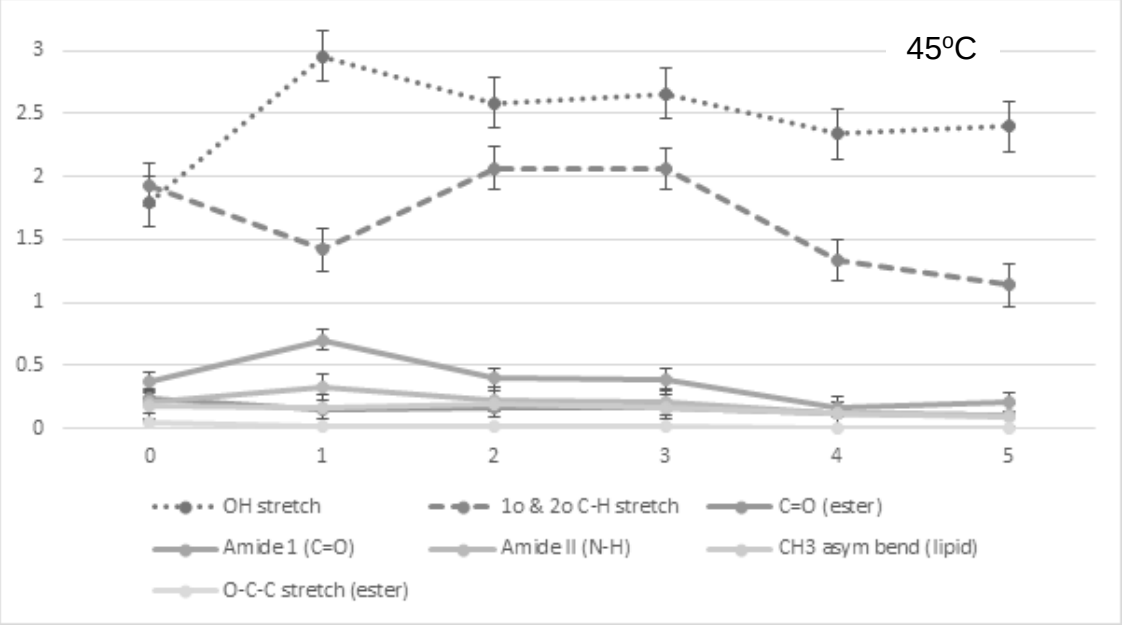
Figure A-1: Absorbance spectra of latent fingermarks as a function of time and temperature for 75ºC, 65 ºC, 55 ºC, 45 ºC, 35 ºC, 25ºC

A.2 Graphical data






55ºC
Abs
Time (hr)
OH stretch
1o & 2o C-H stretch
C=O (ester)
Amide 1 (C=O)
Amide II (N-H)
CH3 asym bend (lipid)
O-C-C stretch (ester)


45ºC
OH stretch
1o & 2o C-H stretch
C=O (ester)
Amide 1 (C=O)
Amide II (N-H)
CH3 asym bend (lipid)
O-C-C stretch (ester)

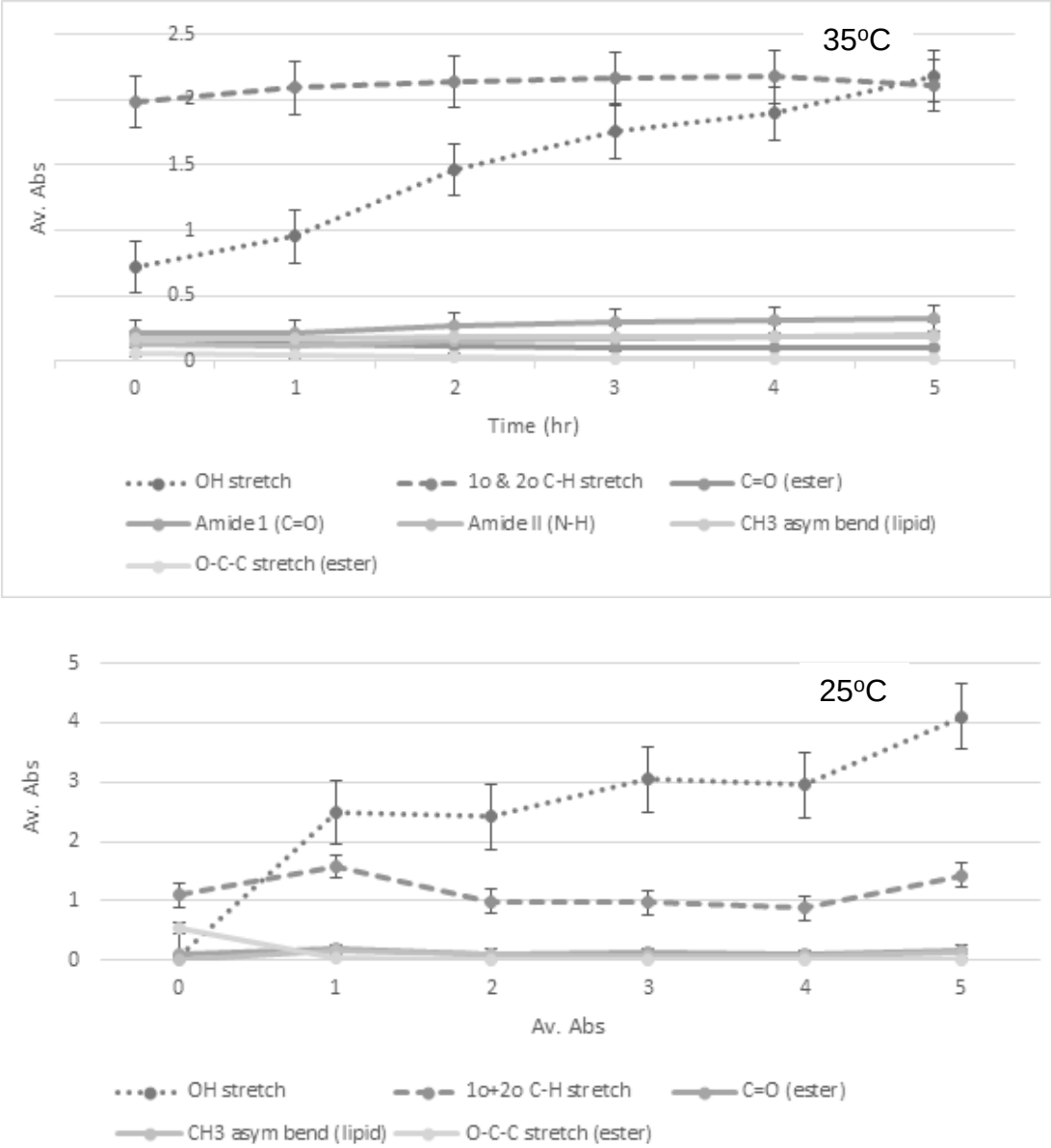


Figure A-2: Changes in IR absorbance of key functional groups over time at 75ºC, 65 ºC, 55 ºC, 45 ºC, 35 ºC, 25ºC

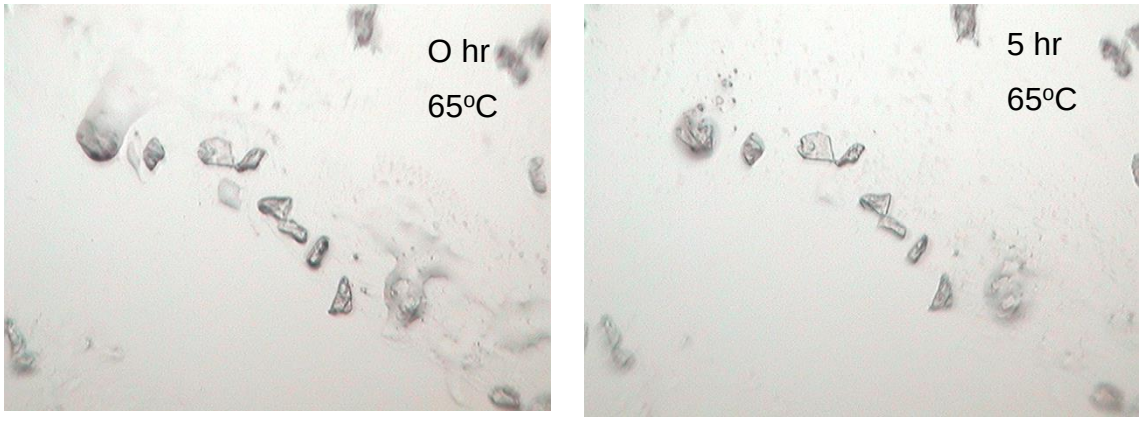


Figure A-3: 100 x 100 µm microscopic image of fingermark deposit at t=0 and t=5 hr at 65°C

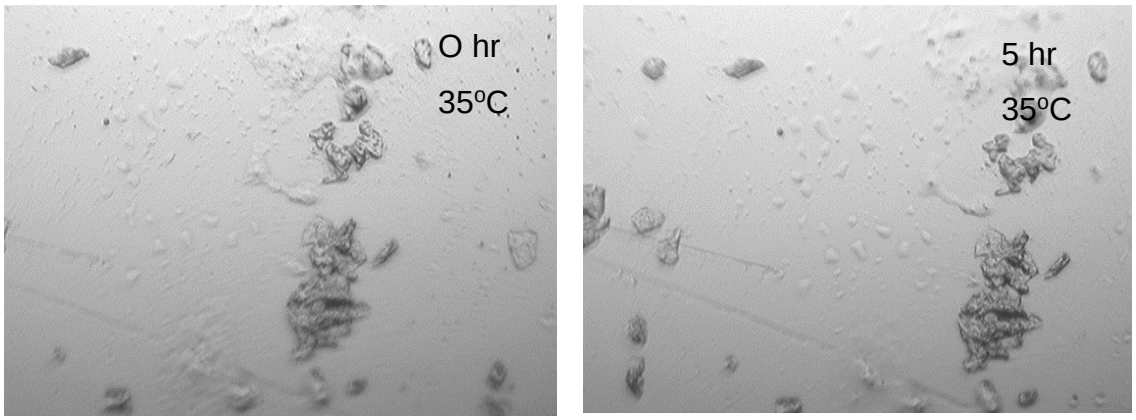


Figure A-4: 100 x 100 µm microscopic image of fingermark deposit at t=0 and t=5 hr at 35°C

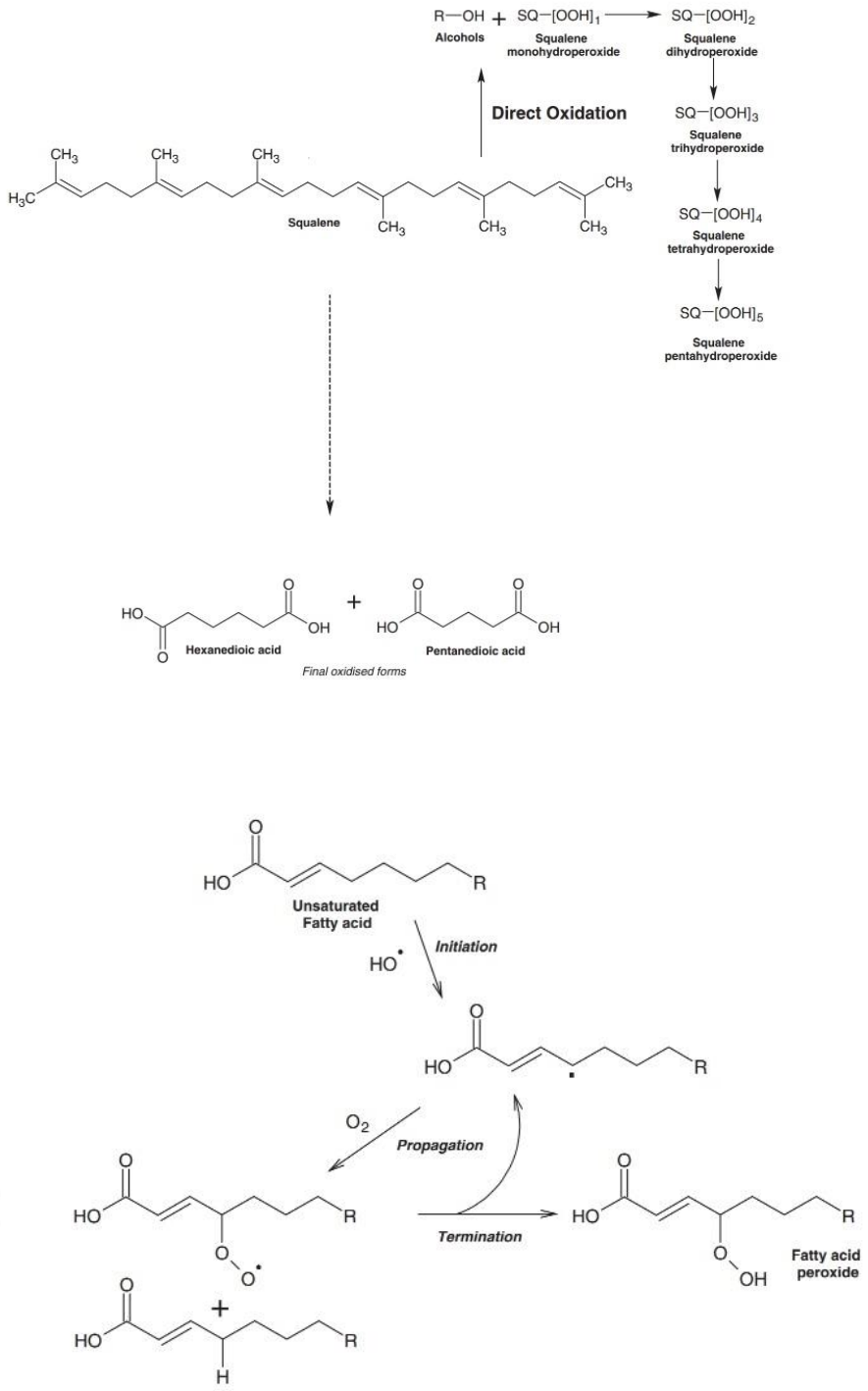


Figure A-5: Possible decomposition pathways contributing to increase in OH stretch band (3250 cm^{-1}) in IR spectra. (Diagrams from Cadd et al. Fingerprint composition and aging: A literature review*. Science and Justice* 55 (2015) 219-238)

Appendix B : Publications

Title	The effect of moderate temperatures on latent fingerprint chemistry
Journal	Applied Spectroscopy
Received	20th November 2016
Accepted	30th January 2017

Title	A study of the intermolecular interactions of lipid components from analogue fingerprint residues
Journal	Science and Justice
Received	25th July 2017
Accepted	20th November 2017

Article

Applied Spectroscopy

Applied Spectroscopy
2017, Vol. 71(9) 2102–2110

DOI: 10.1177/0003702817694902
journals.sagepub.com/home/asp
SAGE

The Effect of Moderate Temperatures on Latent Fingerprint Chemistry

Andrew Johnston and Keith Rogers

Abstract

The effect of moderate temperatures (25–75 °C) on latent fingerprints over a five-hour period was examined using Fourier transform infrared (FT-IR) microspectroscopy. The aim of the study was to detect changes in IR spectra due to any changes in fingerprint chemistry; these results were then compared to pure compounds found in sebum that was subjected to 75 °C for 5 h. Latent fingerprints deposited on CaF_2 microscope slides and placed on a Peltier pump heating stage showed that higher temperatures significantly reduced the quantity of sebaceous compounds after 5 h, whereas temperatures below 45 °C had little effect on the quantity of these compounds over the same time period. Fourier transform infrared microspectroscopy allowed for the real-time detection of changes to the IR spectra and demonstrated an increase in the OH stretch band (3250 cm^{-1}) over 5 h at all temperatures investigated, suggesting various oxidation processes were taking place. Pure samples analyzed included squalene, fatty acids, wax esters, and mixed triglycerides. Unsaturated lipids showed a similar increase in the OH stretch band to the latent fingerprints whereas saturated compounds showed no change over time. This information is required to better understand the effect of moderate temperatures on latent fingerprints and how these temperatures could affect aged print composition.

Keywords

Fingerprints, Fourier transform infrared, FT-IR, microspectroscopy, aging, thermal degradation, decomposition, oxidation

Date received: 20 November 2016; accepted: 30 January 2017

Introduction

The chemical degradation of latent fingerprints post deposition is of great interest to the forensic community. It is well documented that the chemistry of latent prints changes temporally in both adults and children.[1–4] These changes not only affect visualization techniques,[5–7] some being more effective than others on aged prints, but also may potentially provide the basis for an aging tool to establish time since deposition.

The chemical components of a latent fingerprint have been well documented using a variety of analytical techniques[8,9] but the chemical changes that occur over time are less well understood. Previous studies have shown that there are changes in fingerprint chemistry over time and under different conditions. Temporal decomposition[10–15] appears to involve the shortening and degradation of unsaturated lipids including fatty acids (FA), wax esters, triglycerides, and squalene (SQ) due to various oxidation processes.[16–20] In contrast, saturated lipids stay relatively stable over longer time periods (>60 days).[20] A change in unsaturated FA has been observed over time decreasing significantly over a 30-day period.[12,20] This is due to the unsaturated moiety degrading through both aerobic and anaerobic processes and increasing the proportion of unsaturated compounds.[20]

Exposure to light also has a significant impact on fingerprint composition. Studies have shown that exposure to light affects the breakdown mechanisms of various components within fingerprint deposits differently and more rapidly than in dark conditions. Short chain FA and SQ in particular were affected by exposure to light conditions.[12,13] Squalene degradation is supported by the identification of various photo-oxidation mechanisms to produce intermediary products including peroxides, hydroperoxides, and SQ epoxide and the fully oxidized forms being hexanedioic and pentanedioic acid.[19,20] These products are particularly

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prevalent when SQ is exposed to ultraviolet (UV) radiation, such as direct sunlight.[19–21]

Studies into the temporal changes of latent fingerprints, and exposure to light and dark conditions over time, are numerous and have indicated the complex nature of latent fingerprint chemistry. Studies into the effects of other environmental conditions on fingerprint chemistry, such as temperature, are also prevalent but these pyrolytic degradation studies mostly examine the effect of extreme temperatures (>150 °C) from exposure to fires, improvised explosive device (IED) detonations or spent ammunition cartridges.[6,7,22–25] Studies on the effects of more moderate temperature variations on latent prints (from room temperature to 80 °C) are more limited[26] but did demonstrate a general decrease in lipid components.[26] In contrast to previous studies, detailed examination of moderate temperature effects (25–75 °C) could provide significant information into the natural aging processes that occur within latent fingerprints.

Fourier transform infrared (FT-IR) spectroscopy is an emerging but recognized tool for forensic research applications. Previous studies have utilized IR techniques to successfully identify the key components of latent fingerprints,[10,15,26–29] and FT-IR is ideally suited to the analysis of latent prints due to its nondestructive nature and specificity in identifying functional groups in organic compounds. Fourier transform infrared microspectroscopy has proven to be a powerful tool in monitoring temporal changes in latent fingerprints as specific sample areas can be repeatedly analyzed over the duration of an experiment.[26,27]

The aim of this study was to investigate the immediate effects of elevated temperatures (25–75 °C) on the organic compounds within latent fingerprints from 0 to 5 h since deposition. Given the known oxidation processes that occur post deposition of a latent print, this study also aimed to analyze any changes in the IR spectra that could allude to these processes taking place within the first few hours since deposition. The work was strengthened through the examination of temperature effects on individual chemical components found within latent fingerprints thus enabling a more robust interpretation of observed changes. The compounds selected were either the most abundant in their group, e.g., serine shown to be the most abundant amino acid,[30–32] or known to have a significant effect on fingerprint degradation, i.e., SQ.[16–21] The individual components investigated were the steroid precursor SQ, the FA palmitic acid (saturated), linoleic acid (polyunsaturated), the amino acid serine, the ester myristyl myristate, and mixed saturated and unsaturated triglycerides. These compounds were selected to crudely represent the major components within a latent fingerprint and as a representative for each of the essential functional groups under investigation in this study.

Experimental

Sample Preparation

All sample fingerprints were obtained from a single donor (a single donor was used for this small-scale study to maintain a relative consistency between samples, thus any notable observations were likely to be from the variations in temperature and not simply the inter-variability between fingerprints from multiple donors). Hands were first washed and dried thoroughly. The index finger was then drawn from the bridge of the nose, under the eye, to the temple ten times to collect sebaceous secretions and to simulate natural grooming behavior, and placed directly onto a CaF_2 IR microscope slide (10 × 10 mm, Crystran Ltd). All prints were deposited between 09:00 and 10:00 to avoid significant diurnal variations in composition of the latent prints. For each print, the same index finger was used. All fingerprints were destroyed at the end of each day and the process repeated for each additional print; this ensured prints were fresh and analysis was not of inadvertently aged prints.

Heating of Fingerprints

Once the prints were deposited onto the CaF_2 slides, they were placed onto a hollowed-out Peltier pump heat stage and placed under the FT-IR microscope. The temperatures investigated were 25 °C, 35 °C, 45 °C, 55 °C, 65 °C, and 75 °C. Five prints were analyzed at each temperature, five deposits were selected for analysis per print, and spectra were acquired every hour for 5 h at relative humidity.

Fourier Transform Infrared Microspectroscopy

The latent fingerprints were analyzed using a PerkinElmer Spectrum Spotlight 200 FT-IR imaging System equipped with a liquid-nitrogen cooled MCT linear array detector. Data were analyzed using Perkin Elmer software, Spectrum (v.10.02.00), and variations in spectra were processed using peak area calculations. Spectra from fingerprint deposits were collected in transmission mode, the IR beam passing through the slide and the hollowed center of the heat stage, within a 4000–750 cm^{-1} spectral range with ten scans per pixel at 4 cm^{-1} spectral resolution and 10 μm spatial resolution, using a 100 × 100 μm aperture.

Pure Sample Preparation

The preparation consisted of SQ ≥ 98% (Sigma Aldrich, UK), linoleic acid ≥ 99% (Sigma Aldrich, UK), serine ≥ 99% (Sigma Aldrich, UK), myristyl myristate ≥ 99% (Sigma Aldrich, UK), and mixed saturated and unsaturated triglycerides ≥ 98% (Sigma Aldrich, UK). A total of 10 μm of the pure compound was pipetted onto a CaF_2 slide and analyzed in an identical way to the latent fingerprints at 75 °C

for 5 h. Analysis at this temperature allowed for a definitive indication as to whether these compounds were susceptible to thermal degradation.

Results and Discussion

Before any fingerprints were subjected to heat treatment, a control spectrum was obtained from each print. Figure 1 shows a typical FT-IR spectrum of a fresh fingerprint from this study, the key peaks in the range of 3000–1100 cm^{-1}.

This study predominantly focused on variations in quantity of sebaceous materials at different temperatures. These sebaceous compounds correspond to the 2920 cm^{-1} and 2854 cm^{-1} antisymmetric and symmetric C–H stretching modes of CH_2 groups, the C=O stretch of lipids (1741 cm^{-1}), the scissoring mode of CH_2 groups and antisymmetric C–H bending mode of CH_3 groups (1463 cm^{-1}), the CH_3 symmetric bend (1380 cm^{-1}), the C–C–O stretch (1160 cm^{-1}), and the O–C–C stretch (1111 cm^{-1}). Variations in quantity of eccrine secretions were also analyzed, amino acids were observed corresponding to the amide I band (1655 cm^{-1}), and amide II band (1545 cm^{-1}) of secondary amides. The broad O–H stretch band (3250 cm^{-1}) was also observed. It is changes to these sebaceous and eccrine absorption bands that correspond to changes in quantity of the related functional groups and therefore the compounds over time.

Thermally Degraded Fingerprints

Analysis of fingerprints exposed to moderate temperatures indicates significant changes in composition over time.

This study showed a significant reduction in absorbance, and therefore material, of all the key functional groups over the 5 h at 75 °C, 65 °C, and 55 °C with the exception of the amide bands, but no significant reduction at the lower temperatures of 45 °C, 35 °C, and 25 °C (Figures 2 and 3). This suggests that at the higher temperatures, the more volatile sebaceous substituents degrade rapidly, but at the lower temperatures (45 °C, 35 °C, 25 °C) there is less degradation over this short time period; certainly at 35 °C and 25 °C there is no observable change in absorbance between the control and after 5 h. This is to be expected given the far longer time periods required for temporal degradation as observed in the studies mentioned previously, all being performed at room temperature. The reduction in sebaceous material at higher temperatures is understood to be due to the degradation through oxidation of lower molecular weight, volatile unsaturated lipids, including unsaturated FA, triglycerides as well as SQ.[12,19,20] Squalene has been reported to break down rapidly in aged fingerprints reducing in quantity within one day and being almost entirely undetectable within one week,[19] and this process it likely to be accelerated at elevated temperatures.

Figure 4 shows the rate of change in absorbance for the 2920 cm^{-1}, 2854 cm^{-1} C–H stretching modes of CH_2 groups at all six temperatures. Samples heated to 45–75 °C show a significant decrease in C–H absorbance meaning a reduction in material, the fastest reduction in absorbance being at the highest temperatures. The slight increase in the C–H stretching band at 35 °C and 25 °C could be an indication of an initial increase in short chain saturated FA as identified in previous studies,[12] which at higher temperatures evaporate off due to their volatility.

The lack of change in the amide I and II bands (Figure 3) is unsurprising given previous research demonstrating that these compounds do not seemingly undergo any significant photo-degradation over time and thermal degradation is only seen with temperatures in excess of 100 °C.[25]

Most notable in this study was the significant increase in absorbance of the OH stretch band (3250 cm^{-1}) across all

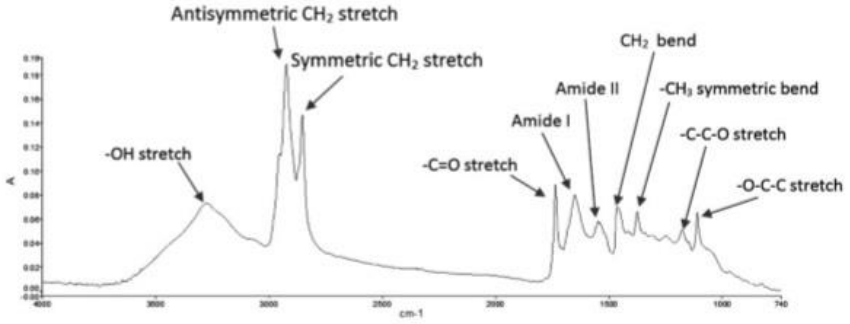

Figure 1. Fourier transform infrared spectrum of a fresh fingerprint from study subject.

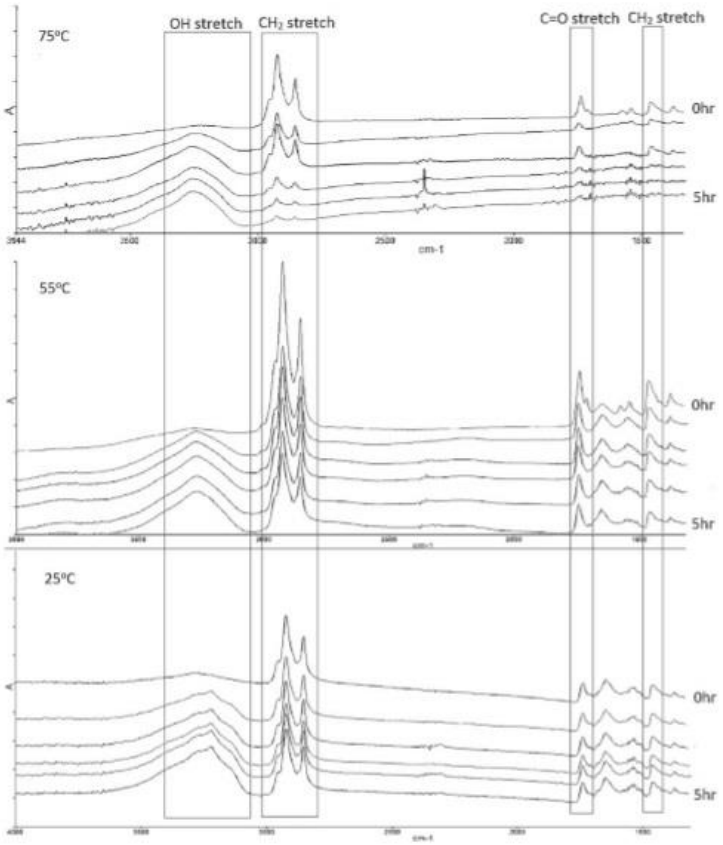

Figure 2. Absorbance spectra of latent fingerprints as a function of time and temperature for 75 °C, 55 °C, and 25 °C.

temperatures. This is in contrast to previous temporal degradation studies that indicate a decrease in OH absorbance over time,[15] thought primarily to be due to a loss of water through evaporation over periods as long as six weeks.

The rapid oxidation of sebaceous compounds such as unsaturated FA, SQ, and glycerides could account for the increase of the OH stretch absorption band. Even accounting for water loss, the net gain being the formation of these hydroxide functional groups due to oxidation mechanisms forming acids, alcohols, and hydroperoxides.[12,19,20] This data suggests that the activation energies required for these processes are below 25 °C as there was no statistical relationship between the rate of change for the increase in the OH stretch band over time and temperature. Given the previously reported rapid degradation of sebaceous compounds such as SQ and certain unsaturated FA, it is likely that these processes require a low activation energy for oxidation to occur. Although the temperatures tested in this study do not influence the rate of change of the OH absorption band, higher temperatures (65 °C and 75 °C) do appear to limit the quantity of hydroxide functional groups being formed when compared to lower temperatures (Figure 3). This is most likely to be due to a more rapid loss of water at these higher temperatures as well as a loss of the more volatile organic compounds.

Pure Compounds

If specific sebaceous secretions are responsible for these oxidation processes and the resulting increase in the OH stretch band of the IR spectra, it is important to identify which compounds within these secretions are the main

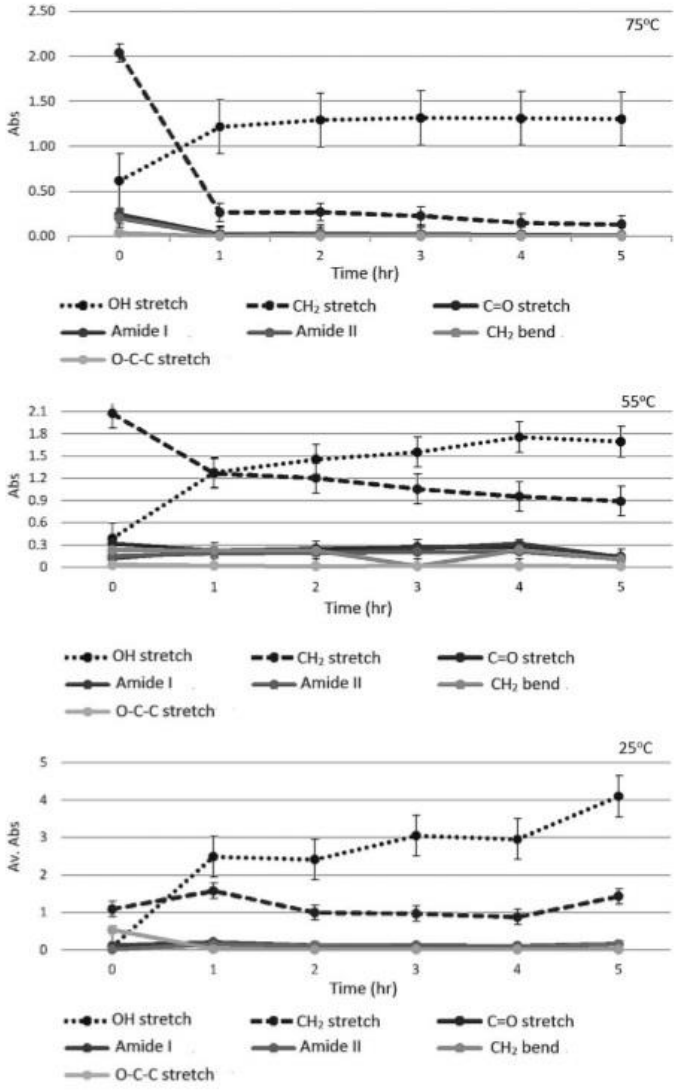

Figure 3. Changes in IR absorbance of key functional groups over time at 75 °C, 55 °C, and 25 °C.

contributors to this change. As mentioned above, previous studies have demonstrated that certain unsaturated lipids decompose rapidly and could therefore contribute to the increase in absorbance of the OH band at 3250 cm^{-1}. This study looked at the major components of a latent fingerprint to identify which pure compounds, such as SQ, or compound groups, such as FA, most readily decomposed and contributed to the increase in absorbance of the OH band. Compounds primarily found in sebaceous secretions were selected, namely SQ, palmitic acid (saturated

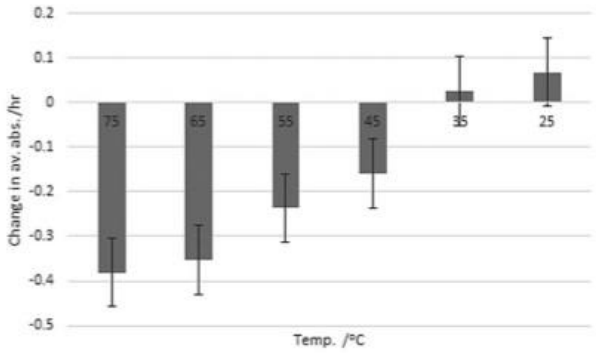

Figure 4. Rate of change of the C-H stretch band absorbance at 75 °C, 65 °C, 55 °C, 45 °C, 35 °C, and 25 °C.

FA), linoleic acid (polyunsaturated FA), myristyl myristate (wax ester), and mixed triglycerides, as well as the amino acid serine, an eccrine secretion. Figure 5 shows an example spectra of the changes in the OH band over time for SQ.

Of the six compound groups investigated in this study (Figure 6), SQ, the mixed triglycerides, and linoleic acid showed significant changes in the OH stretch region at 3250 cm^{-1}. Serine, palmitic acid, and myristyl myristate showed no significant changes over the duration of the experiment. These data suggest that thermal degradation processes impact unsaturated sebaceous compounds significantly, but not saturated sebaceous compounds or amino acids. The increase in the OH stretch region at 3250 cm^{-1} in SQ supports previous studies reporting the rapid oxidation of this compound to produce intermediaries such as alcohols, mono- and polyhydroperoxides, and finally hexanedioic acid and pentanedioic acid. The mixed triglycerides analyzed showed an increase in the OH stretch band over the 5 h, indicating degradation processes in action, although not to the same extent as SQ (Figure 6). Some previous research has suggested possible triglyceride degradation mechanisms resulting in both saturated and unsaturated FA,[33,34] the unsaturated FA then likely go through further oxidation thus continuing to increase the OH band. In a latent fingerprint, it is likely that triglyceride degradation processes would be increased due to the presence of cholesterol which has been shown to affect triglyceride decomposition.[34] Of the FA analyzed, linoleic acid (Figure 6), a polyunsaturated FA, showed a rapid initial increase in the OH stretch region in the first hour, then little change over the remaining 4 h. This suggests rapid oxidation occurring on this unsaturated compound perhaps, as previously proposed, to produce FA peroxides through aerobic degradation.[36] It is worth remembering, however, that polyunsaturated FAs only make up ~2% of sebaceous secretions[17,36] so it is unlikely

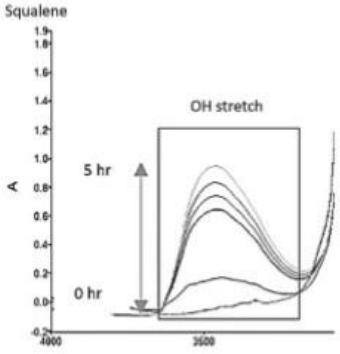

Figure 5. Infrared spectra of the 3250 cm^{-1} OH stretch band of pure squalene heated to 75 °C for 5 h.

that these compounds would have a significant effect on the increase of the OH stretch band of a latent fingerprint. Monounsaturated FAs would also degrade aerobically however and they make up ~48% of sebum.[17,36] The OH stretch band of palmitic acid, a saturated FA, showed no significant variation in this study. This is consistent with temporal degradation studies reporting that C16 FAs, such as palmitic acid, remain relatively stable over a 60-day period.[20] The OH stretch band of myristyl myristate also showed no significant changes over the duration of this study, again consistent with previous studies showing that these saturated lipids are not significantly affected by temporal degradation due to a lack of functional groups.[17]

Serine, the only non-sebaceous compound analyzed in this study showed no correlation between temperature and changes in the OH stretch region. As mentioned above previous studies have shown that significant thermal

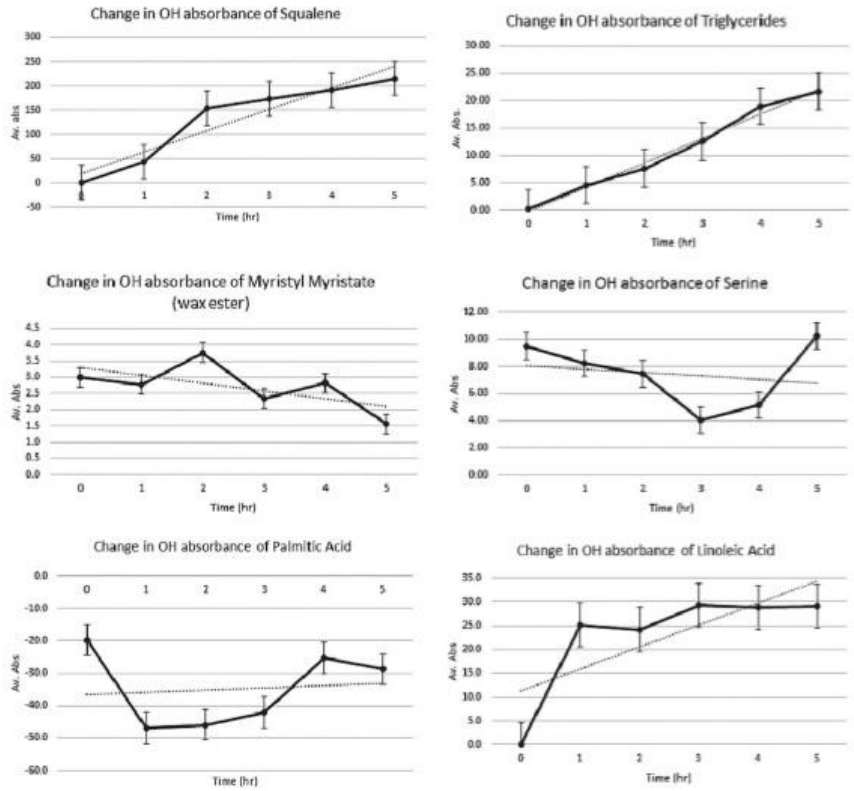

Figure 6. The increase in OH absorbance (3250 cm^{-1}) of SQ, mixed triglycerides, myristyl myristate, serine, palmitic acid, and linoleic acid at 75 °C over 5 h.

degradation of amino acids occurs at 100 °C and higher[24,25] due to their low volatility, so it is not surprising that there were no significant changes in the 3250 cm^{-1} OH band in this study.

Conclusions

The research presented within this paper suggests that the oxidation processes that occur during thermal degradation of latent fingerprints could be similar, if not identical, to that of temporal aging at a constant ambient temperature as shown in previous studies,[12,16,19,20] but at a faster rate. It is reasonable to assume that latent fingerprints at a crime scene would be subject to varying diurnal temperatures from the time of deposition to the time of analysis by law enforcement agencies, varying from hours to days. This study demonstrates the dynamic nature of chemical decomposition within a fingerprint after just a few hours, higher temperatures causing more rapid degradation of the unsaturated and volatile lipids, which could impact crime scene work. Developing fingerprints that have been exposed to higher diurnal temperatures could be more challenging, particularly when using those developing agents that rely on lipophilic interactions. Further work is required to investigate the extent and consistency of these degradation processes using multiple donors to identify trends in decomposition, as well as the effect of different substrates on fingerprint chemistry. Future studies to better understand the degradation products that are likely to occur at varying temperatures could lead to better development techniques, more suited to prints subjected to higher diurnal temperatures.

The pure materials analyzed in this study translate to the compounds involved in the degradation processes of

natural fingerprints. The analysis of pure samples that are present in sebum showed that SQ, in particular, but also triglycerides and mono/poly-unsaturated FA, are most likely to be the cause of an increase in the OH stretch band at 3250 cm^{-1} over time. This is likely to be due to various intermediary and complete oxidation processes. Future work accounting for individual variability would provide a better understanding of the interactions between these compounds and how each component affects the stability of the other.

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Conflict of Interest

The authors report there are no conflicts of interest.

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References

1. M.V. Buchanan, K. Asano, A.I. Bohanon. "Chemical Characterization of Fingerprints". Proc. SPIE 2941, Forensic Evidence Analysis and Crime Scene Investigation, 89. 1997. 2941: 89–95. doi:10.1117/12.266300.
2. D.K. Williams, C.J. Brown, J. Bruker. "Characterization of Children's Latent Fingerprint Residues by Infrared Microspectroscopy: Forensic Implications". Forensic Sci. Int. 2011. 206(1–3): 161–165.
3. D. Noble. "Vanished into Thin Air: The Search for Children's Fingerprints". Anal. Chem. 1995. 67(13): 435A–438A.
4. K.M. Antoine, S. Mortazavi, L.M. Miller. "Chemical Differences are Observed in Children's Versus Adults' Latent Fingerprints as a Function of Time". J. Forensic Sci. 2010. 55(2): 513–518.
5. K. Sampson, W. Sampson. "Recovery of Latent Prints from Human Skin". J. Forensic Identif. 2005. 55(3): 362–385.
6. A.G. Brown, D. Sommerville, B.J. Reedy, R.G. Shimmon, M. Tahtouh. "Revisiting the Thermal Development of Latent Fingerprints on Porous Surfaces: New Aspects and Refinements". J. Forensic Sci. 2009. 54(1): 114–121.
7. A. Dominick, N. Nicdaeid, S. Bleay, V. Sears. "The Recoverability of Fingerprints on Paper Exposed to Elevated Temperatures, Part 1: Comparison of Enhancement Techniques". J. Forensic Identif. 2010. 59(3): 325–339.
8. S. Cadd, M. Islam, P. Manson, S. Bleay. "Fingerprint Composition and Aging: A Literature Review". Sci. Justice. 2015. 55(4): 219–238.
9. M.J. Bailey, N.J. Bright, S. Hinder, R.S. Croxton, S. Francese, et al. "Chemical Characterization of Latent Fingerprints by Matrix-Assisted Laser Desorption Ionization, Time-of-Flight Secondary Ion Mass Spectrometry, Mega Electron Volt Secondary Mass Spectrometry, Gas Chromatography/Mass Spectrometry, X-ray Photoelectron Spectroscopy, and Attenuated Total Reflection Fourier Transform Infrared Spectroscopic Imaging: An Intercomparison". Anal. Chem. 2012. 84(20): 8514–8523.
10. A. Girod, L. Xiao, B. Reedy, C. Roux, C. Weyermann. "Fingermark Initial Composition and Aging Using Fourier Transform Infrared Microspectroscopy (M-FT-IR)". Forensic Sci. Int. 2015. 254: 185–196.
11. F. Cuthbertson, J.R. Morris. "The Chemistry of Fingerprints". AWRE Report No. 013/69. United Kingdom Atomic Energy Authority, 1969.
12. N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells. "Changes in the Lipid Composition of Latent Fingerprint Residue with Time after Deposition on a Surface". Forensic Sci. Int. 2005. 154(2–3): 224–239.
13. R.S. Croxton, M.G. Baron, D. Butler, T. Kent, V.G. Sears. "Variation in Amino Acid and Lipid Composition of Latent Fingerprints". Forensic Sci. Int. 2010. 199: 93–102.
14. A. Girod, R. Ramotowski, C. Weyermann. "Composition of Fingermark Residue: A Qualitative and Quantitative Review". Forensic Sci. Int. 2012. 223(1–3): 10–24.
15. P. Fritz, W. Van Bronswjik, K. Lepkova, et al. "Infrared Microspectroscopy Studies of the Chemical Composition of Latent Fingermark Residues". Microchem. J. 2013. 111: 40–46.
16. C. Weyermann, C. Roux, C. Champod. "Initial Results on the Composition of Fingerprints and its Evolution as a Function of Time by GC/MS Analysis". J. Forensic Sci. 2011. 56(1): 102–108.
17. R. Ramotowski. "Composition of Latent Print Residues". In: H. Lee, R. Gaensslen (eds) Advances in Fingerprint Technology. London: CRC Press, 2001, pp.63–104.
18. R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese. "Study of Latent Fingermarks by Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry Imaging of Endogenous Lipids". Rapid Commun Mass Spectrom. 2009. 23(19): 3031–3039.
19. K.A. Mountfort, H. Bronstein, N. Archer, S.M. Jickells. "Identification of Oxidation Products of Squalene in Solution and in Latent Fingerprints by ESI-MS and LC/APCI-MS". Anal. Chem. 2007. 79(7): 2650–2657.
20. G.M. Mong, C.E. Petersen, T. Clauss. "Advanced Fingerprint Analysis Project - Fingerprint Constituents". Vol PNNL-13019. Pacific Northwest National Laboratory: U.S. Department of Energy 1999.
21. M. Picardo, C. Zompetta, C. De Luca, et al. "Squalene Peroxides May Contribute to Ultraviolet Light-Induced Immunological Effects". Photodermatol. Photoimmunol. Photomed. 1991. 8(3): 105–110.
22. C.M. Girelli, B.J.M. Lobo, A.G. Cunha, J.C.C. Freitas, F.G. Emmerich. "Comparison of Practical Techniques to Develop Latent Fingermarks on Fired and Unfired Cartridge Cases". Forensic Sci. Int. 2015. 250: 17–26.
23. W.W. Harper. "Latent Fingerprints at High Temperatures". J. Crim. Law Criminol. 1938. 29(4): 580–583.
24. A. Richmond-Aylor, S. Bell, P. Callery, K. Morris. "Thermal Degradation Analysis of Amino Acids in Fingerprint Residue by Pyrolysis GC-MS to Develop New Latent Fingerprint Developing Reagents". J. Forensic Sci. 2007. 52(2): 380–382.
25. G. De Paoli, S.A. Lewis, E.L. Schuette, L.A. Lewis, R.M. Connatser, et al. "Photo- and Thermal-Degradation Studies of Select Eccrine Fingerprint Constituents". J. Forensic Sci. 2010. 55(4): 962–969.
26. C. Ricci, P. Phiriyavityopas, N. Curum, K.L. Andrew Chan, S. Jickells, et al. "Chemical Imaging of Latent Fingerprint Residues". Appl. Spectrosc. 2007. 61(5): 514–522.
27. D.K. Williams, R.L. Schwartz, E.G. Bartick. "Analysis of Latent Fingerprints Deposits by Infrared Microspectroscopy". Appl. Spectrosc. 2004. 58(3): 313–316.
28. N.J. Crane, E.G. Bartick, R.S. Perlman, S. Huffman. "Infrared Spectroscopic Imaging for Noninvasive Detection of Latent Fingerprints". J. Forensic Sci. 2007. 52(1): 48–53.
29. A. Hemmila, J. McGill, D. Ritter. "Fourier Transform Infrared Reflectance Spectra of Latent Fingerprints: A Biometric Gauge for the Age of An Individual". J. Forensic Sci. 2008. 53(2): 369–376.
30. B. Hadorn, F. Hanimann, P. Anders, H. Curtius, R. Halverson. "Free Amino-Acids in Human Sweat from Different Parts of the Body". Nature. 1965. 215(99): 416–417.
31. P. Hamilton. "Amino-Acids on Hands". Nature. 1965. 205: 284–285.

32. J. Oro, H. Skewes. "Free Amino-Acids on Human Fingers: The Question of Contamination in Microanalysis". Nature. 1965. 207(1): 1042–1045.
33. A. Srivastava, R. Prasad. "Triglycerides-Based Diesel Fuels". Renew. Sust. Energy Rev. 2000. 4(2): 111–113.
34. L. Iuliano. "Pathways of Cholesterol Oxidation via Non-Enzymatic Mechanisms". Chem. Phys. Lipids. 2011. 164(6): 457–468.
35. B.B. Dent, S.L. Forbes, B.H. Stuart. "Review of Human Decomposition Processes in Soil". Environ. Geol. 2004. 45(4): 576–585.
36. A. Girod, C. Weyermann. "Lipid Composition of Fingermark Residue and Donor Classification Using GC/MS". Forensic Sci. Int. 2014. 238: 68–82.
37. T. Atherton, R. Croxton, M. Baron, J. Gonzalez-Rodriguez, L. Gámiz-Gracia, A.M. García-Campaña. "Analysis of Amino Acids in Latent Fingerprint Residue by Capillary Electrophoresis-Mass Spectrometry". J. Sep. Sci. 2012. 35: 2994–2999.

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A study of the intermolecular interactions of lipid components from analogue fingerprint residues

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ABSTRACT

A compositionally simplified analogue of a latent fingermark was created by combining single representatives of each major component of a natural fingermark. Further modified analogues were also produced each having one component removed. The aim of this study was to investigate the intermolecular interactions that occurred within these analogue samples using Fourier Transform Infrared (FT-IR) Microspectroscopy. FT-IR microspectroscopy showed that the absence of squalene and cholesterol significantly restricted the interactions between the other organic constituents within the analogue samples. Investigating the intermolecular interactions of organic compounds within a simplified analogue solution could indicate corresponding interactions that occur within natural fingermarks. These potential interactions could go on to be the target of further investigation of latent fingermark chemistry, and ultimately contribute to a better understanding of the aging processes and degradation mechanisms that take place post-deposition.

1. Introduction

The dynamic nature of fingermark chemistry post-deposition is a complex process, yet of great interest to the forensic community. Numerous studies have examined the aging of fingermarks, the oxidation mechanisms that take place, both short and long term [1,2], and the resulting end products [2–5], [8], [9–12]. However, an in-depth understanding of fingermark degradation processes remains limited. Various studies have investigated the breakdown of key components within a fingermark such as squalene [1–3], [9,13], fatty acids [2–5,7], cholesterol [4], [9–11], [14] and amino acids [15,16], although very little research has studied the intermolecular interactions between these constituents and their impact on each other. Ultimately, a better understanding of these intermolecular interactions within a fingermark will allow for improved modelling of the fingermark aging process.

Cholesterol and its oxidation products have a significant effect on the decomposition of triglycerides and fatty acids [10], indicating that lipid stability is influenced by these intermolecular interactions within latent fingermarks. Auto-oxidation, oxidation in the absence of enzymatic catalysis, of cholesterol by free radicals and hydroperoxides to form oxysterols is an established degradation mechanism [10], although evidence of these oxidation products are yet to be found in latent fingermarks. It has also been shown that the decomposition of cholesterol can be accelerated by both triglycerides and fatty acids [11,17,18]. This suggests a form of positive feedback wherein cholesterol affects the decomposition of triglycerides resulting in a mixture of saturated and unsaturated fatty acids [6]. This increase in fatty acid concentration could then increase the rate of decomposition of cholesterol.

Squalene is an unsaturated steroid precursor that has received a great deal of attention in previous studies of fingermark degradation mechanisms and the aging of fingermarks [1–3], [9,13]. In latent fingermarks squalene degrades rapidly over time through direct oxidation and photo-oxidation mechanisms. Measurable reductions in concentration can be demonstrated within 24 h following deposition and it is almost undetectable after a week [1]. Although this degradation is dependent on light conditions as squalene can still be detected in fingermarks up to 33 days after deposition when stored in the dark [3]. During degradation various intermediary and complete oxidation products have previously been identified [1,2]. Of particular note is the formation of various hydroperoxides as squalene decomposes through direct oxidation. These hydroperoxides then undergo thermolysis with homolytic scission of the peroxide bond, yielding hydroxyl radicals [18]. Both hydroperoxides and free radicals, such as hydroxyl radicals produced from squalene degradation, would then impact cholesterol breakdown and impact the degradation of triglycerides and fatty acids.

This investigation utilized Fourier transform infrared (FT-IR) microspectroscopy, is a recognised tool for forensic research applications. Previous studies using IR techniques have successfully identified the key components of latent fingermarks [19–25]. FT-IR is ideally suited to

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the analysis of latent prints due to its non-destructive nature and specificity in identifying functional groups in organic compounds. Of particular relevance to this study FT-IR spectroscopy lends itself to studying the intermolecular interactions within fingermarks as a major contributor to IR band width is the strength of intermolecular interactions. Broader peaks being a function of stronger intermolecular interactions over a wider range and therefore a large number of energy states.

The antisymmetric and symmetric C−H stretch of the CH_2 groups at 3000–2850 cm^{-1} within a fingermark are not truly decoupled due to the slight variation in chemical environment of the differing species present. Intermolecular forces such as hydrogen bonding, dipole/dipole interactions, and London Dispersive Forces all combine to increase organic molecular interactions [26–32]. These interactions will dictate the rate of reaction and therefore the molecular decomposition in the presence of (in particular) long chain unsaturated species. These intermolecular interactions affect the range of vibrational modes of the C−H stretch region of CH_2 groups, increasing variations in bond distance and changing the spring constant [31–35]. The more intermolecular interactions occurring the broader the antisymmetric and symmetric vibrational modes and the coupling effect visible on the spectrum (Fig. 1). Fewer intermolecular interactions perturb the range of vibrational modes of the C−H stretch mode at 3000–2850 cm^{-1}, minimising the coupling effect of the antisymmetric and symmetric C−H peaks, and producing two distinct peaks at 3000–2850 cm^{-1}.

The aim of this study was to generate a simplified chemical analogue of a fingermark, comprised of a single representative of the major components, in order to better understand the interactions between these compounds. Studying these intermolecular interactions could ultimately enable a better understanding of their effect on the aging of fingermarks post-deposition. Once an acceptable analogue was developed and the IR spectra compared to that of sebaceous-loaded fingermarks, further analogues were developed, each with one component removed, and IR spectra were then obtained. This allowed assessment of how this removed component affected the interactions of the others.

Replicates of latent fingermarks have previously been developed for both research and commercial purposes [5,36,37] to provide a 'standardised' deposition model, and with more complexity than the analogue samples developed for this study. The objective of this study however was not to create a complete replicate of a latent fingermark, but to deliberately develop a much simplified composition that allowed for fundamental analysis of any critical intermolecular interactions that may occur. More complex replicates involving hundreds of compounds would have made analysis of specific intermolecular interactions near impossible, or at least far less conclusive. Another reason why such a simplified composition was used in this study was because, as stated previously [37], synthetic solutions can behave differently to natural fingermarks, and the more complex the solution the more potential there is for inconclusive variability in the results. A primary use for replicate solutions is for identifying effective fingerprint development reagents. The International Fingerprint Research Group (IFRG) have stated that replicates are useful for fundamental research and initial study of molecular interactions, but are not appropriate for optimisation or validation trials [36], and have warned caution about using (particularly) synthetic lipid solutions for direct evaluation with natural latent fingermarks, although this was specifically regarding fingerprint development reagents [38].

It is evident therefore that a degree of caution must be employed when using a simplified analogue solution to study the chemical interactions within fingermarks, and certainly no direct comparisons between the two can be made. This study therefore aimed to provide a general indication of the intermolecular interactions that *may* occur within latent fingermarks, and thus present a target for further work to look for these potential interactions in latent fingermarks and their impact on fingerprint degradation.

2. Experimental

2.1. Sample preparation

2.1.1. Analogue 'fingermark' preparation

The composition of the analogue solution was based on the principal compounds observed during previous studies of latent fingermarks [2,5,19,37,39,40]. Where there was significant variation in the literature regarding the concentration of a component, an average of all available data was used.

Sebaceous and organic eccrine secretions were selected for the analogue. The sebaceous secretions comprised a sterol, sterol precursor, a fatty acid, mixed triglycerides (100 mg triacetin, tributyrin, tricaproin, tricaprylin, tricaprin, all equal amounts by weight), and a wax ester. The eccrine secretions were composed from an amino acid and lactic acid. For simplicity, the most abundant compound within each family (i.e. amino acid, fatty acid, wax ester) was selected to represent the compounds within that family. For example, serine, being the most abundant amino acid [41–43] was selected to represent all amino acids, and palmitic acid, the most abundant fatty acid, represented all fatty acids [2,3] (Unsaturated fatty acids, although more likely to have an impact on intermolecular interactions due to a targetable functional

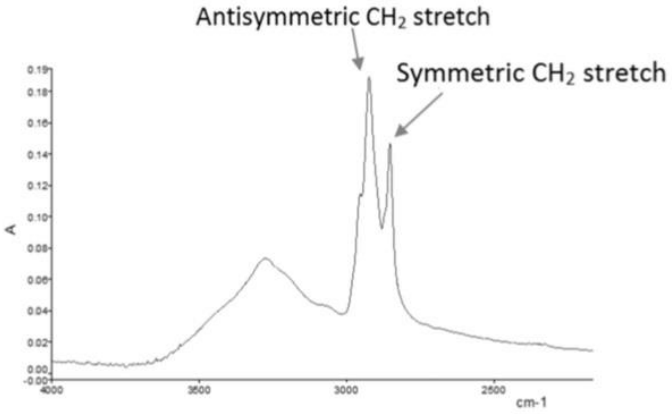

Fig. 1. Coupling effect of the antisymmetric and symmetric C−H stretch of CH_2 group.

Table 1
Compounds and concentrations selected to create analogue fingermark composition.

Compound	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Concentration	11.2 mg L^{-1}	1.1 mg L^{-1}	15.4 mg L^{-1}	2.5 mg/L^{-1}	10 mg L^{-1}	42.1 mg L^{-1}	29.9 mg L^{-1}

Table 2
Composition of the eight analogue 'fingermark' samples.

Sample 1 'complete'	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 2	–	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 3	Squalene	–	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 4	Squalene	Cholesterol	–	Serine	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 5	Squalene	Cholesterol	Palmitic acid	–	Lactic acid	Mixed triglycerides	Myristyl myristate
Sample 6	Squalene	Cholesterol	Palmitic acid	Serine	–	Mixed triglycerides	Myristyl myristate
Sample 7	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	–	Myristyl myristate
Sample 8	Squalene	Cholesterol	Palmitic acid	Serine	Lactic acid	Mixed triglycerides	–

group [43], were not included in this study due to lack of data on origin or quantity).

This abundancy approach was also reflected in the concentrations of each compound that made up the analogue solution, which represented the total concentration of that compound group within a latent fingermark. For example, the concentration of serine in the analogue was 1.45 mgL^{-1}, equivalent to the typical total concentration of amino acids observed in eccrine sweat [5,12]. The compounds selected and their concentrations are shown in Table 1 (all compounds ≥ 98%, obtained from Sigma Aldrich, UK).

Table 2 shows the composition of each sample. Each sample was made up to a 1 L solution with distilled water. Sample 1, comprised of all the constituents shown in Table 1, was used as the control and samples 2–7 all having one of the compounds in Table 1 removed.

During formulation each sample was heated to 37 °C to replicate body temperature and to force any solid compounds into solution with the addition of 50 mgL^{-1} of the emulsifier oleyl alcohol. Immediately after mixing 10 μL of the analogue samples were pipetted onto CaF_2 Infrared (IR) microscope slides (10 mm × 10 mm, Crystran Ltd) and placed on a Peltier pump heat stage to maintain 'body temperature' and placed under the IR microscope for analysis. Each sample was analysed 20 times and an average spectra obtained.

2.1.2. Sebaceous-loaded fingermarks

All fingermarks were obtained from a single donor (38-year-old male, wearing no cosmetics) for this study to maintain a relative consistency between samples, and the spectra obtained were consistent with data from previous studies. Hands were first washed and dried thoroughly. The index finger was then drawn from the bridge of the nose, under the eye to the temple ten times to collect sebaceous secretions and to simulate natural grooming behaviour, and placed directly onto a CaF_2 IR microscope slide (10 mm × 10 mm, Crystran Ltd) at an average pressure of 74,800PA. All prints were deposited between 9 am and 10 am to avoid significant diurnal variations in composition of the latent fingermark and analysed immediately. For each print the same index finger was used 20 samples were analysed at room temperature and atmospheric pressure and an average spectra obtained from 10 randomly chosen locations per print.

2.2. FT-IR microspectroscopy

The sebaceous-loaded fingermarks and the analogue compositions were analysed using a PerkinElmer Spectrum™ Spotlight 200 FT-IR imaging System equipped with a liquid-nitrogen cooled MCT linear array detector. Data was analysed using Perkin Elmer software, Spectrum® (v10.02.00), variations in spectra were processed using peak area calculations. Spectra were collected in transmission mode, the IR beam passing through the slide, within 4000 to 750 cm^{-1} spectral range with 10 scans per pixel at 4 cm^{-1} spectral resolution and 10 μm spatial resolution, using a 100 × 100 μm aperture.

3. Results and discussion

Prior to analysis of the analogue samples, spectra from sebaceous-

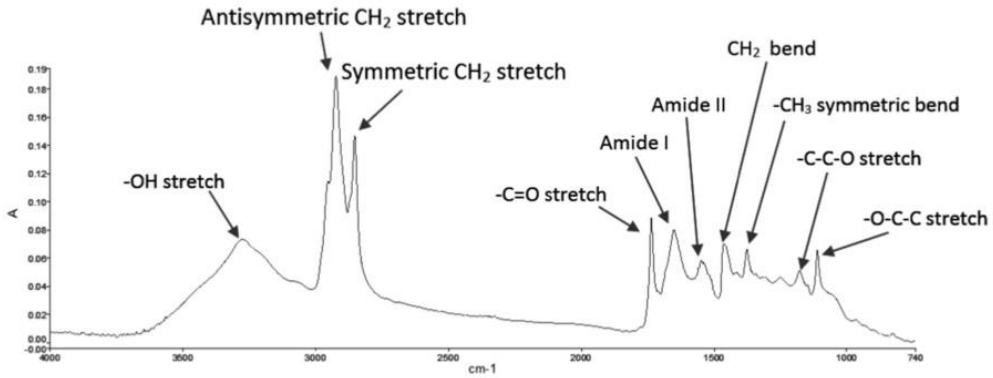

Fig. 2. Typical FTIR spectrum of a fresh fingermark.

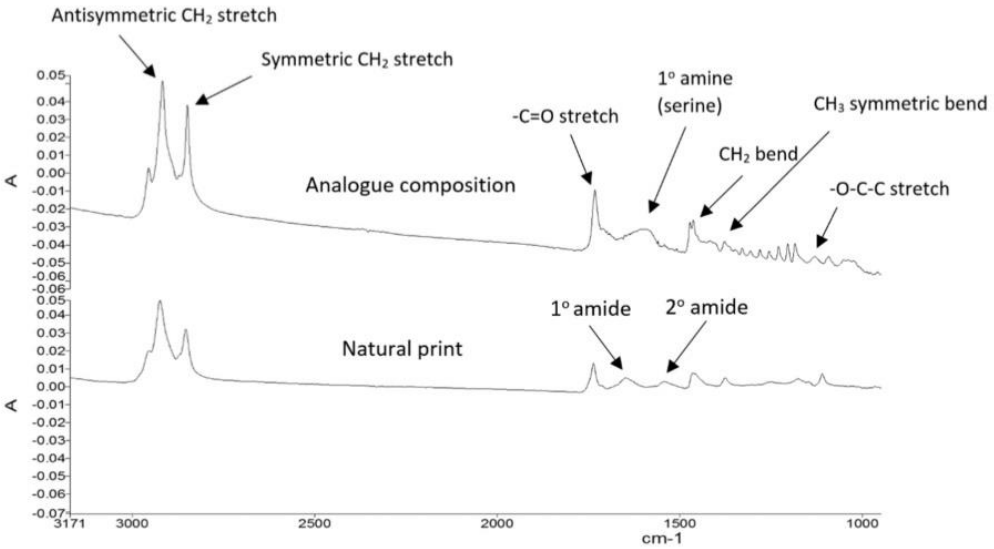

Fig. 3. FT-IR spectral comparison between the analogue sample and a natural fingermark.

loaded fingermarks were obtained to serve as a spectral comparison to the complete analogue sample. Fig. 2 shows a typical FT-IR spectrum of a fresh fingermark from this study, the key peaks ranging from 3000 cm^{-1} to 1100 cm^{-1}.

This study predominantly focused on the interactions between sebaceous materials within a fingermark. These sebaceous compounds correspond to the 2920 $cm^{-1}$and 2854 cm^{-1} antisymmetric and symmetric C–H stretching modes of CH_2 groups, the C=O stretch of lipids (1741 cm^{-1}), the scissoring mode of CH_2 groups and antisymmetric C–H bending mode of CH_3 groups (1463 cm^{-1}), the CH_3 symmetric bend (1380 cm^{-1}), the C–C–O stretch (1160 cm^{-1}), and the O–C–C stretch (1111 cm^{-1}). Variations in quantity of some eccrine secretions were also analysed, amino acids were observed corresponding to the amide I band (1655 cm^{-1}), and amide II band (1545 cm^{-1}) of secondary amides. It is these sebaceous and eccrine absorption bands that correspond to quantity of the related functional groups and therefore the compounds.

3.1. Analogue samples

The 'complete' analogue sample demonstrated an accurate IR spectral representation of a sebaceous-loaded fingermark (Fig. 3).

The spectra of the 'complete' analogue sample correspond well to that of a sebaceous-loaded fingermark, all the major peaks were present in relatively accurate quantities, the exception being the 1° amine peak at ~1600 cm^{-1} in the analogue sample. This band represents the amino acid serine that was a representative for all the amino acids present in a natural fingermark. This band is not present in natural fingermarks, instead the 1° and 2° amide peaks from peptides and proteins dominate this region. Due to the stability and unreactive nature of amino acids within latent fingermarks, only degrading at > 100 °C, and not undergoing photo-degradation [13,15] it was felt that this would have minimal effect on the dynamics of the analogue composition.

Once it was established that the IR spectra of the 'complete' analogue sample accurately represented that of the sebaceous-loaded fingermark further samples were created, each with a component from the 'complete' analogue removed. As would be expected removing a component from the sample had a corresponding effect on the spectrum for that sample. For example, removing serine resulted in an absence of the corresponding absorption band at ~1600 cm^{-1}. Equally, removing the wax ester myristyl myristate resulted in an observable reduction in the antisymmetric and symmetric CH_2 stretching modes at 2920 $cm^{-1}$and 2854 cm^{-1}, the C=O stretch mode at 1736 cm^{-1}, and the CH_2 bend at 1462 cm^{-1}, although the peak distribution within the spectra remained the same. Removing lactic acid from the sample had

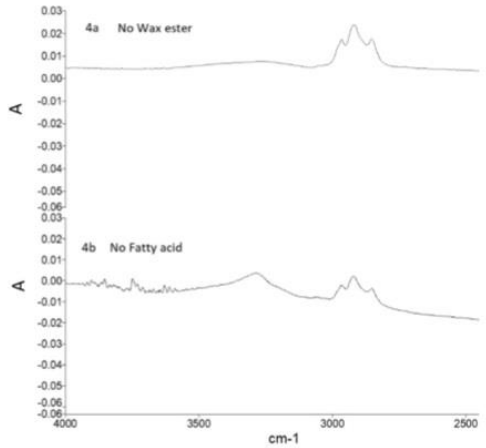

Fig. 4. The antisymmetric and symmetric C–H stretch modes of CH_2 groups for analogue samples with the wax ester removed (4a), and with the fatty acid removed (4b).

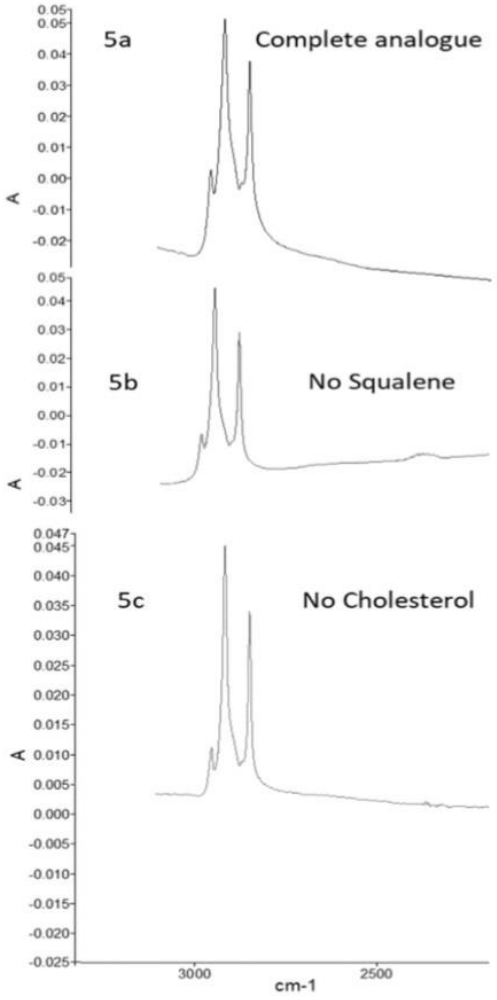

Fig. 5. The antisymmetric and symmetric C–H stretch modes of CH_2 groups for a 'complete' analogue sample (5a), with squalene removed (5b), and with cholesterol removed (5c).

no observable effect on the spectra, this was to be expected given the small amount of the compound within the sample, also the functional groups within lactic acid, namely the hydroxide and carbonyl groups, are present in a many of the other components within the analogue sample as well as in natural fingermarks.

The 2920 $cm^{-1}$and 2854 cm^{-1} antisymmetric and symmetric C–H stretching modes of CH_2 groups are a combination of all the organic components within a sample. Removing key constituents such as wax esters or fatty acids had an impact on the height of these absorbance bands, and therefore quantity, as would be expected. As discussed earlier a cause of variation in band width are intermolecular interactions, which, in this study did not significantly vary from the 'complete' analogue sample in the absence of the wax ester, fatty acid, amino acid, mixed triglycerides or lactic acid, although peak height was affected, the coupling effect of the C–H stretch mode was not (Fig. 4). However, the absence of the steroid precursor squalene and the sterol cholesterol modified the coupling effect of the C–H stretching modes significantly (Fig. 5).

It could be reasoned that the absence of cholesterol and squalene would reduce peak height and therefore overall peak area simply due to a reduction in quantity of the sample analysed. Peak height however is not significantly different in the absence of these two components and therefore changes in peak area are directly proportional to peak width and therefore bond diversity (Fig. 5). Additionally, a reduction in peak height alone is not sufficient to explain this decrease in overall peak area in the absence of squalene or cholesterol. These components made up a small proportion of the overall sample, and as Fig. 5 shows, the absence of these components had no observable effect on peak height and therefore concentration. Also, it is only in the absence of squalene and cholesterol that such distinct C–H stretching modes, with minimal coupling, are generated.

The antisymmetric and symmetric C–H stretching modes of CH_2 groups in the absences of squalene and cholesterol (Fig. 5b and c) show two distinct peaks with little overlap when compared to the coupling of the same peaks from the 'complete' analogue (Fig. 5a). This indicates that the intermolecular interactions in the absence of squalene and cholesterol are significantly reduced in these analogue samples.

Peak area calculations of the antisymmetric and symmetric C–H stretching modes of CH_2 groups quantify the spectral data and show that analogue samples, in the absence squalene or cholesterol, have a significantly lower absorption area between 2780 and 2995 cm^{-1} than that of the other analogue samples (Fig. 6).

The analogue samples investigated in this study suggest that the presence of both squalene and cholesterol are essential in driving intermolecular interaction within these samples. This could provide a focus for further research looking at the importance of these compounds within the dynamic environment of latent fingermark chemistry, and if squalene and cholesterol have an integral role in the aging processes within natural fingerprints.

As mentioned previously cholesterol can influence the degradation of triglycerides producing both saturated and unsaturated fatty acids [10]. The absence of this sterol could significantly slow the degradation of these other lipid compounds and reduce the quantity of degradation end products such as fatty acids. In a natural fingermark, saturated fatty acids appear to increase during the first few days after deposition [3]. The hydroperoxides and hydroxyl radicals produced during the oxidation of squalene are also likely to affect the degradation of cholesterol and unsaturated fatty acids, and thus have a concomitant effect upon

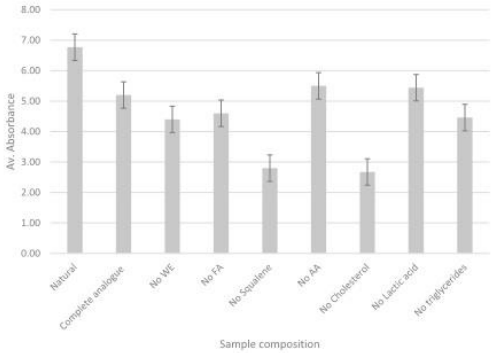

Fig. 6. Average absorbance area of C–H stretching modes at 2780–2995 cm^{-1} of analogue samples and natural fingermarks.

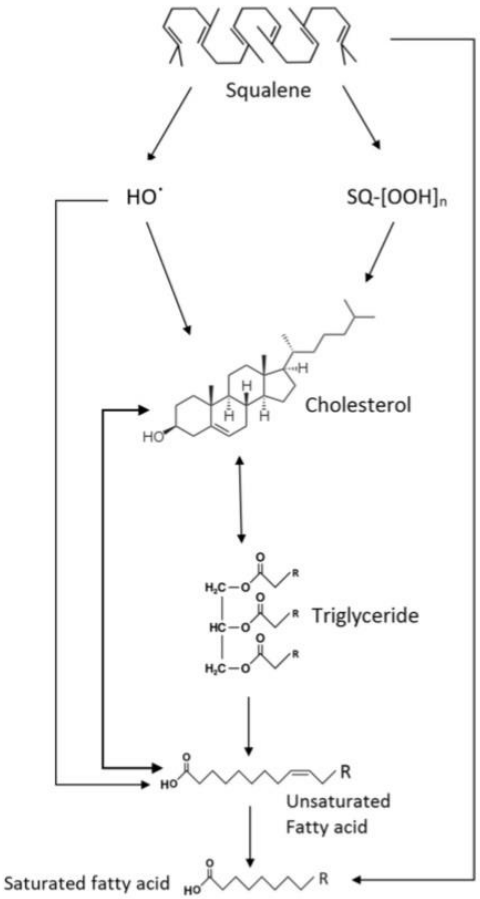

Fig. 7. Suggested interaction pathway of lipid compounds within a latent fingermark.

triglyceride decomposition and the initial increase in saturated fatty acids (Fig. 7).

Assuming that squalene and cholesterol are essential in driving the interactions between the various sebaceous compounds present in a latent fingermark, then this may, at least in part, explain why children's latent fingermarks are more volatile and tend to 'vanish' instead of adopting the temporal degradation of adult prints [44–46]. Children's latent fingermarks contain very little squalene so interactions between this steroid precursor and other sebaceous compounds (such as unbranched fatty acids) would be limited. Reduced interactions would mean that degradation of these other sebaceous compounds (particularly the more volatile ones) to more stable states would be reduced leaving them more prone to evaporation. The sebaceous components of young children also contain significantly lower levels of triglycerides than that of adults, so the breakdown of cholesterol and fatty acids through interactions with triglycerides (and vice versa) to more stable end products would also be inhibited.

4. Conclusions

The research presented in this paper demonstrates that squalene and cholesterol affect the intermolecular interactions within the analogue samples studied, this could indicate the importance of these two compounds in influencing intermolecular interactions between lipids within latent fingermarks, and provides a hypothesis for studying these interactions within natural fingermarks. The degradation processes that occur in latent fingermarks are directed by these interactions and only by studying them will it be possible to understand the complex and dynamic nature of fingermark aging. Future studies using analogue compositions could investigate whether these analogue samples age in similar ways to natural fingermarks by identifying known degradation products that occur in natural latent fingermarks. Analogue compositions of latent fingermarks will always be crude representations of the myriad of interactions that occur within natural fingermarks, but ongoing investigation into these simplified models, utilising various analytical techniques, could direct research that ultimately provides a better understanding of the fingermark aging process.

References

[1] K.A. Mountfort, H. Bronstein, N. Archer, S.M. Jickells, Identification of oxidation products of squalene in solution and in latent fingermarks by ESI-MS and LC/APCI-MS, Anal. Chem. 79 (7) (2007) 2650–2657.
[2] G.M. Mong, C.E. Petersen, T.R.W. Clauss, Advanced Fingerprint Analysis Project - Fingerprint Constituents, vol. PNNL-13019, U.S. Department of Energy, Pacific Northwest National Laboratory, 1999.
[3] N.E. Archer, Y. Charles, J.A. Elliott, S. Jickells, Changes in the lipid composition of latent fingerprint residue with time after deposition on a surface, Forensic Sci. Int. 154 (2–3) (2005) 224–239.
[4] R.S. Croxton, M.G. Baron, D. Butler, T. Kent, V.G. Sears, Variation in amino acid and lipid composition of latent fingerprints, Forensic Sci. Int. 199 (2010) 93–102.
[5] R. Ramotowski, Composition of latent print residues, Advanced in Fingerprint Technology, 2nd ed., CRC Press, London, 2001.
[6] A. Srivastava, R. Prasad, Triglycerides-based diesel fuels, Renew. Sust. Energ. Rev. 4 (2) (2000) 111–113.
[7] B.B. Dent, S.L. Forbes, B.H. Stuart, Review of human decomposition processes in soil, Environ. Geol. (2004) 576–585.
[8] R. Wolstenholme, R. Bradshaw, M.R. Clench, S. Francese, Study of latent fingermarks by matrix-assisted laser desorption/ionisation mass spectrometry imaging of endogenous lipids, Rapid Commun. Mass Spectrom. 23 (19) (2009) 3031–3039.
[9] C. Weyermann, C. Roux, C. Champod, Initial results on the composition of fingerprints and its evolution as a function of time by GC/MS analysis, J. Forensic Sci. 56 (1) (2011) 102–108.
[10] L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, Chem. Phys. Lipids 164 (6) (2011) 457–468.
[11] S.K. Kim, W.W. Nawar, Oxidative interactions of cholesterol with triacylglycerols, J. Am. Oil Chem. Soc. 68 (12) (1991) 931–934.
[12] A. Girod, R. Ramotowski, C. Weyermann, Composition of fingermark residue: a qualitative and quantitative review, Forensic Sci. Int. 223 (1–3) (2012) 10–24.
[13] T. Atherton, R. Croxton, M. Baron, J. Gonzalez-Rodriguez, L. Gámiz-Gracia, A.M. García-Campaña, Analysis of amino acids in latent fingerprint residue by capillary electrophoresis-mass spectrometry, J. Sep. Sci. 35 (2012) 2994–2999.
[14] A. Stadtman, A. Cherkes, C. Anfinsen, Studies on the microbiological degradation of cholesterol, J. Biol. Chem. 206 (2) (1954) 511–523.
[15] A. Richmond-Aylor, S. Bell, P. Callery, K. Morris, Thermal degradation analysis of amino acids in fingerprint residue by pyrolysis GC-MS to develop new latent fingerprint developing reagents, J. Forensic Sci. 52 (2) (2007) 380–382.
[16] G. De Paoli, S.A. Lewis, E.L. Schuette, L.A. Lewis, R.M. Connatser, T. Farkas, Photo- and thermal-degradation studies of select eccrine fingerprint constituents, J. Forensic Sci. 55 (4) (2010) 962–969.
[17] S.K. Kim, W.W. Nawar, Parameters influencing cholesterol oxidation, Lipids 28 (10) (1993) 917–922.
[18] L.L. Smith, Cholesterol autoxidation 1981–1986, Chem. Phys. Lipids 44 (2–4) (1987) 87–125.
[19] A. Girod, L. Xiao, B. Reedy, C. Roux, C. Weyermann, Fingermark initial composition and aging using Fourier transform infrared microscopy (μ-FTIR), Forensic Sci. Int. 254 (2015) 185–196.
[20] P. Fritz, W. van Bronswjik, K. Lepkova, S.W. Lewis, K.F. Lim, D.E. Martin, L. Puskar, K.F. Lim, D.E. Martin, L. Puskar, K.F. Lim, D.E. Martin, L. Puskar, Infrared microscopy studies of the chemical composition of latent fingermark residues, Microchem. J. 111 (2013) 40–46.
[21] C. Ricci, S.G. Kazarian, Collection and detection of latent fingermarks contaminated with cosmetics on nonporous and porous surfaces, Surf. Interface Anal. 42 (5) (2010) 386–392.
[22] D.K. Williams, R.L. Schwartz, E.G. Bartick, Analysis of latent fingerprints deposits by infrared microspectroscopy, Appl. Spectrosc. 58 (3) (2004) 313–316.
[23] N.J. Crane III, E.G. Bartick, R.S. Perlman, S. Huffman, Infrared spectroscopic

imaging for noninvasive detection of latent fingerprints, J. Forensic Sci. 52 (1) (2007) 48–53.
[24] A. Hemmila, J. McGill, D. Ritter, Fourier transform infrared reflectance spectra of latent fingerprints: a biometric gauge for the age of an individual, J. Forensic Sci. 53 (2) (2008) 369–376.
[25] A. Johnston, K. Rogers, The effect of moderate temperatures on latent fingerprint chemistry, Appl. Spectrosc. 71 (9) (2017) 2102–2110.
[26] N.B. Colthup, L.H. Daly, S.E. Wiberley, N.B. Colthup, L.H. Daly, S.E. Wiberley, Chapter 4 – The Vibrational origin of group frequencies, Introduction to Infrared and Raman Spectroscopy, 1990, pp. 171–213.
[27] G. Socrates, Infrared Characteristic Group Frequencies. Tables and Charts, Second ed., John Wiley & Son Ltd., 1994, pp. 34–41.
[28] F.M. Fowkes, Donor-acceptor interactions at interfaces, J. Adhes. 4 (2) (1972) 155–159.
[29] N. Israelachvili, J.N. Tabor, The measurement of Van Der Waals dispersion forces in the range 1.5 to 130 nm, Proceedings of the Royal Society A, 1972, pp. 19–38.
[30] J. Israelachvili, Intermolecular and Surface Forces, 3rd ed., Elsevier, 2011.
[31] X. Li, Q. Pan, J. Chen, S. Liu, A. He, C. Liu, Y. Wei, K. Huang, L. Yang, J. Feng, Y. Zhao, Y. Xu, Y. Ozaki, I. Noda, J. Wu, Asynchronous orthogonal sample design scheme for two-dimensional correlation spectroscopy (2D-COS) and its application in probing intermolecular interactions from overlapping infrared (IR) bands, Appl. Spectrosc. 65 (8) (2011) 901–917.
[32] J. Chen, Q. Bi, S. Liu, X. Li, Y. Liu, Y. Zhai, Y. Zhao, L. Yang, Y. Xu, I. Noda, J. Wu, Double asynchronous orthogonal sample design scheme for probing intermolecular interactions, J. Phys. Chem. 116 (45) (2012) 10904–10916.
[33] N.B. Colthup, L.H. Daly, S.E. Wiberley, N.B. Colthup, L.H. Daly, S.E. Wiberley, Chapter 5 – Methyl and Methylene groups, Introduction to Infrared and Raman Spectroscopy, 1990, pp. 215–233.
[34] A.L. Steber, J.L. Neill, D.P. Zaleski, B.H. Pate, A. Lesarri, R.G. Bird, V. Vaquero-Vara, D.W. Pratt, Structural studies of biomolecules in the gas phase by chirped-pulse Fourier transform microwave spectroscopy, Faraday Discuss. (2011) 227–242.
[35] B.K. Sharma, Instrumental Methods of Chemical Analysis, Twenty Fourth edition, GOEL, Meerut, 2005.
[36] J. Almog, A.A. Cantu, C. Champod, T. Kent, C. Lennard, Guidelines for the assessment of fingermark detection techniques. International Fingerprint Research Group (IFRG), J. Forensic Identif. 64 (2) (2014) 174–200.
[37] A. Girod, C. Weyermann, Lipid composition of fingermark residue and donor classification using GC/MS, Forensic Sci. Int. 238 (2014) 68–82.
[38] S. Zadnik, W. Van Bronswijk, A. Frick, P. Fritz, S.W. Lewis, Fingermark simulants and their inherent problems: a comparison with latent fingermark deposits, J. Forensic Identif. 63 (5) (2013) 593–608.
[39] M.J. Bailey, N.J. Bright, S. Hinder, B.N. Jones, R.P. Webb, R.S. Croxton, S. Francese, L.S. Ferguson, R. Wolstenholme, S. Jickells, B.J. Jones, J.J. Ojeda, S.G. Kazarian, S. Bleay, Chemical characterization of latent fingerprints by matrix-assisted laser desorption ionization, time-of-flight secondary ion mass spectrometry, mega electron volt secondary mass spectrometry, gas chromatography/mass spectrometry, X-ray photoelectron spectroscopy, and attenuated total reflection Fourier transform infrared spectroscopic imaging: an intercomparison, Anal. Chem. 84 (20) (2012) 8514–8523.
[40] S. Cadd, M. Islam, P. Manson, S. Bleay, Fingerprint composition and aging: a literature review, Sci. Justice 55 (4) (2015) 219–238.
[41] P. Hamilton, Amino-acids on hands, Nature 205 (1965) 284–285.
[42] J. Oro, H. Skewes, Free amino-acids on human fingers: the question of contamination in microanalysis, Nature 207 (1) (1965) 1042–1045.
[43] B. Hadorn, F. Hanimann, P. Anders, H. Curtius, R. Halverson, Free amino-acids in human sweat from different parts of the body, Nature 215 (99) (1965) 416–417.
[44] K.M. Antoine, S. Mortazavi, L.M. Miller, A.D. Miller, K.M. Antoine, S. Mortazavi, A.D. Miller, L.M. Miller, Chemical differences are observed in children's versus adults' latent fingerprints as a function of time, J. Forensic Sci. 55 (2) (2010) 513–518.
[45] D.K. Williams, C.J. Brown, J. Bruker, Characterization of children's latent fingerprint residues by infrared microspectroscopy: forensic implications, Forensic Sci. Int. 206 (1–3) (2011) 161–165.
[46] D. Noble, Vanished into thin air. The search for children's fingerprints, Anal. Chem. 67 (13) (1995) 435A–438A.